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Factors Affecting Carcinogenesis

III. The Effect of Hydrogenation of Lipid Solvents on Carcinogenesis by 3,4-Benzpyrene

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The cocarcinogenic or anticarcinogenic effect of various lipid solvents, when used as vehicles for the subcutaneous injection into mice of a standard dose of 0.3 mgm. of 3,4-benzpyrene, has been the subject of two previous publications from this Laboratory (3, 12). The effects then observed might be explained on the basis of either physical or chemical factors. Physical factors possibly important would include the rate of diffusion of benzpyrene within the solvent, the surface tension and rate of dispersal of the solvent within the tissues, the state of aggregation of the solvent, and similar properties; whereas a chemical explanation would assume the presence of a substance (or substances) that would react directly with either the carcinogen or some cell constituent or would influence indirectly, by positive or negative catalysis, some reaction essential in carcinogenesis. The physical properties of two oily solvents such as sesame oil (cocarcinogenic) and the liquid fraction of "mouse fat" (anticarcinogenic) seem to be so similar as to make the purely physical theory unlikely. The interest and attractiveness of the "solvent effects" lie in the rather greater probability of the chemical theory, since, if it were possible to identify the components responsible, they might provide a clue for the exploration of the still unknown reactions leading up to carcinogenesis.

With the chemical theory as our working hypothesis it was our object to track down the component that conferred on mouse fat its protective power. Various possibilities suggested themselves. As the preparation of mouse fat previously used (3) was made by solvent extraction of whole mouse carcasses it contained, in addition to neutral fats, other lipids: notably phospholipins and free and esterified cholesterol. The anticarcinogenic effect of ox brain lipids (3) as well as of purified lecithin and cephalin (12) supported the special significance of these admixtures. Another possible difference in the chemical composition of mouse fat and sesame or arachis oils was the presence in the former of certain unsaturated fatty acids, such as arachidonic or clupanodonic acid, peculiar to animal fats. As cod liver oil, which is a particularly rich source of these polyethenoid fatty acids, was devoid of anticarcinogenic properties (12) it seemed unlikely that the anticarcinogenic principle of mouse fat would reside in the unsaturated fatty acid fraction. However, it appeared desirable to obtain more conclusive evidence on this point, and it was therefore decided to compare the solvent effects of mouse fat and cod liver oil before and after hydrogenation. Such an investigation seemed especially valuable in view of the results of Leiter and Shear (8), who found a correlation between retardation of tumor induction and increasing saturation of the long-chain fatty acids in a triglyceride solvent. To facilitate the study of the saturation effect uncomplicated by the effect of lipid admixtures a sample of mouse fat was prepared from excised adipose tissue.

As in the second paper of this series (12), the observation of tumor incidence was supplemented by measurement of the rate of elimination of benzpyrene with each of the solvents used. Our previous attempts in this direction were not entirely satisfactory, owing to insufficient control of leakage. We have succeeded in improving this factor considerably by various measures described below, especially by discarding the pellet technic, with which the quantitative retention of the implant was too uncertain.

EXPERIMENTAL

Preparation of Mouse Fat

The dissected abdominal and subcutaneous adipose tissue of 60 freshly killed mice was extracted 3 times with about 600 ml. of acetone. The combined acetone extracts were dried over $CaCl_2$, filtered, and evaporated under reduced pressure in an atmosphere of nitrogen. The residue consisted of 107 gm. mouse fat (preparation *B*), which contained 0.167 per cent of total cholesterol, estimated colorimetrically after saponification according to Ireland (7), and approximately 0.002 per cent of P, estimated colorimetrically by the method of Berenblum and Chain (1), corresponding to a phospholipin content of about 0.05 per cent.

Hydrogenation

A. Cod liver oil.—50 gm. of medicinal cod liver oil were dissolved in 100 ml. of ethyl acetate and hydrogenated at room temperature and a hydrogen pressure of 4 atmospheres in the presence of 50 mgm. of Adams' catalyst under vigorous shaking. The solution turned to a semisolid after 1 hour, then quickly became a very stiff paste. The hydrogen uptake was completed after 2.3 hours.

Iodine value before hydrogenation: 170.7
" " after " : 21.4

B. Mouse fat.—A solution of 50 gm. in 100 ml. of ethyl acetate was first treated in the same way as cod liver oil, for 9 hours with shaking, and for an additional 11 hours standing under pressure. The solution was still a mobile liquid, and the iodine value was decreased by only 10 per cent. Hydrogenation was therefore repeated at 60° C., with an additional 50 mgm. of catalyst and a hydrogen pressure of 1.1 atmospheres for 7 hours. Even after this treatment hydrogenation had proceeded only to the extent of 34 per cent, as shown by the iodine value. Hydrogenation was therefore repeated a third time, now at 100° C. in the absence of solvent. Again a fresh sample of catalyst was used and the mixture was stirred under atmospheric pressure of hydrogen for 3 hours, when the hydrogen uptake had ceased. On cooling, the fat solidified completely.

Iodine value before hydrogenation: 81.7
" " after " : 4.0

Both fats were purified after hydrogenation by dissolving them in warm ether, boiling with activated charcoal, filtration, and evaporation.

Injection Technic

As the hydrogenated fats were high-melting solids it was necessary to dissolve them in an equal amount of tricaprylin at about 60° C. At this temperature the solution could be readily injected under the skin, but solidified almost immediately afterwards. There was therefore no danger of primary leakage (3) with these two solvents. The two unhydrogenated fats, which were oils, were also diluted with an equal volume of tricaprylin; thus the degree of saturation was the only difference in the two parallel series with natural and hydrogenated fats. It appeared that these oily solutions tended to leak through the skin puncture caused by the hypodermic needle. This factor was studied in careful preliminary experiments by visual inspection in ultraviolet light and by recovery estimations carried out on batches of 4 mice each 24 hours after injection. Previously we had used needles 5 cm. long, which were introduced near the tail and pushed under the skin along the whole length of the animal towards the neck. Leakage was greatly reduced by using thinner though shorter needles (No. 17), and by injecting into the skin pocket of the left axilla. No apparent advantage was gained by injecting from head to tail, as recommended by Berenblum and Schoental (2). Even with this improved technic occasional leakage occurred. It was found that this could be further reduced by immobilizing the animals for the first hours after injection with an anesthetic (intraperitoneal injection of Numal Roche, diluted 1 in 30 with water, 0.2 ml. per 10 gm. of body weight). During anesthesia the animals were kept covered with cotton wool in a heated room. Twenty-four hours after injection they were inspected in ultraviolet light, and those showing any considerable patches of fluorescence of the fur were rejected.

3,4-Benzpyrene was dissolved in tricaprylin to give a concentration of 2 mgm./ml. and this solution was then mixed with equal parts of the various fat fractions. Each mouse received 0.3 ml. of the mixture, containing 0.3 mgm. of benzpyrene.

Male mice of mixed stock were used for the experiments, and 45 were usually injected in each series; 30 of these were kept for observation of the tumor incidence and the remainder used for analysis of the rate of elimination of benzpyrene, one animal of each series being killed each week for this purpose.

All mice were inspected daily for the first few days, twice weekly during the first month, and later, when the danger of ulceration had largely passed, once weekly.

The benzpyrene content of the whole mouse was estimated according to Weil-Malherbe (10). Amounts of benzpyrene between 0.05 and 3 μgm. were estimated by the two-condition technic of Miller and Baumann (9) after chromatographic purification, but using pure oxygen instead of air (11).

Observations During Latent Period

Immediately following the injection, hard lumps of the hydrogenated fat were present at the injection site when the solvent was hydrogenated cod liver oil or hydrogenated mouse fat. In the former series this lump provoked a severe inflammatory reaction in a few days, which surrounded the pellet with a soft, fluctuant mass; this was eventually followed in more than half the animals in this series by ulceration. In the remainder the pellet was sometimes absorbed or dispersed into small fragments, but occasionally it persisted for several months. The hydrogenated mouse fat formed very hard, flat disks, which lay loosely under the skin without provoking any noticeable reaction from the surrounding tissues; in most animals of this series residues of the injected material persisted until death.

Whereas the injected natural cod liver oil formed diffuse deposits of semisolid, yellow, greasy, insoluble material, the natural mouse fat led to the formation of encapsulated oil cysts that were usually palpable as soft lumps under the skin. These solvent deposits were often observed at autopsy long after complete disappearance of benzpyrene could be inferred on the basis of the elimination analyses. In the natural mouse fat series, sarcomas were sometimes seen to originate from the wall of an oil cyst. There were a few ulcers in the natural cod liver oil series, whereas in the natural mouse fat series ulcers were entirely absent with specimen *B;* but with mouse fat *A* ulcers were present in one-quarter of the injected mice, and this incidence of ulceration was much increased after the mouse fat had been stored for a long time (Table II).

All mice with ulcers were dismissed from the experiment, although some of them subsequently developed tumors, thus indicating that ulceration does not necessarily lead to complete ejection of the carcinogen.

RESULTS

Incidence of Neoplasms

Effectual total number of mice.—From the total number of mice in each group originally injected, all mice that died before the appearance of the first tumor in that particular group have been eliminated, together with those in which ulceration or sloughing had occurred at any stage of the experiment. The balance is described in the tables as the effectual total.

In order to make possible the comparison of tumor incidence in different series, the results have been graphically shown as percentage responses; but the actual numbers are given in the tables, since they are necessary for estimating the probable significance of any differences observed.

Cod liver oil and hydrogenated cod liver oil.—The 50 per cent solutions of these two solvents in tricaprylin were injected into 30 and 60 mice respectively. The larger number of mice were used for the hydrogenated oil in order to allow for the high proportion of animals that had to be rejected owing to ulceration. This is shown in Table I, which also includes data for the incidence of local tumors at the 20th, 40th, and 50th week after injection. Fig. 1 shows the time course of the incidence. It will be noted that the mixture of cod liver oil and tricaprylin behaved very much the same as did the undiluted cod liver oil in our previous experiment (12); in the latter, the total incidence of tumors was about the same in tricaprylin (34 per cent) and in cod liver oil (37 per cent), but the latent period was diminished in cod liver oil. In the present experiment the total incidence was 35 per cent, and the latent period with the unsaturated oil much shorter than with the hydrogenated material. The total incidence was also diminished to 21 per cent in the latter.

Table I: Incidence of Tumors; Fats Before and After Hydrogenation

Solvent (Mixed with equal volume of tricaprylin)	No. of mice	No. died before first tumor appeared	No. with ulcers	Effectual total	Total local sarcomas					
					20 weeks		40 weeks		60 weeks	
					No.	Per cent	No.	Per cent	No.	Per cent
Mouse fat *B* + tricaprylin	30	5	0	25	12	48	15	60	17	68
Hydrogenated mouse fat *B*	30	0	6	24	12	50	22	92	22	92
Cod liver oil + tricaprylin	30	5	5	20	3	15	7	35	7	35
Hydrogenated cod liver oil	60	9	32	19	0	0	3	16	4	21

A notable feature with hydrogenated cod liver oil was the unusually frequent occurrence of distant tumors. These were; 2 leukemias; 1 hepatoma, histologically nonmalignant; 1 malignant mesenteric tumor; and 1 malignant neoplasm of the lung. These do not appear among "local tumors" in Table I, but one lymphosarcoma arising near the injection site has been included. No such distant tumors occurred with the unhydrogenated oil, either in the present series or in the previous one.

Mouse fat and hydrogenated mouse fat.—The injection of benzpyrene in either of these solvents, which were again mixed with an equal volume of tricaprylin, led to a high incidence of neoplasms (Table I and Fig. 1). The induction period tended to be a little shorter with the natural fat, but the final incidence was higher

with the hydrogenated material (92 per cent) than with the unhydrogenated fat (68 per cent). Only one distant tumor, a hepatoma, occurred and this was with the hydrogenated fat.

Behavior of different samples of mouse fat.—Since it was found that mouse fat *B* diluted with an equal volume of tricaprylin had no inhibitory action on carcinogenesis, whereas the original sample (mouse fat *A*) was previously (3) shown to be anticarcinogenic, the next step was to test mouse fat *B* undiluted with tricaprylin. At the same time the remainder of the original sample (mouse fat *A*), which had been kept in the laboratory for 3 years since it was first tested, was again injected. Thirty mice were used for each series. The incidence of tumors is shown in Table II and Fig. 2, both of which include for comparison the data originally observed by us (3) for mouse fat *A*. The tumor incidence with pure tricaprylin as solvent is also shown as an example of the behavior of a synthetic glyceride.

The most obvious point arising from these experiments is that mouse fat *B*, prepared from dissected adipose tissue, has no inhibitory effect on carcinogenesis, whether it is dissolved in tricaprylin or not; the two curves are closely similar (Fig. 2). The final tumor incidence with stale mouse fat *A* is the same as in pure tricaprylin. The latent period was shortened with mouse fat *B* compared with tricaprylin, whereas it was prolonged by mouse fat *A*, even after 3 years' storage.

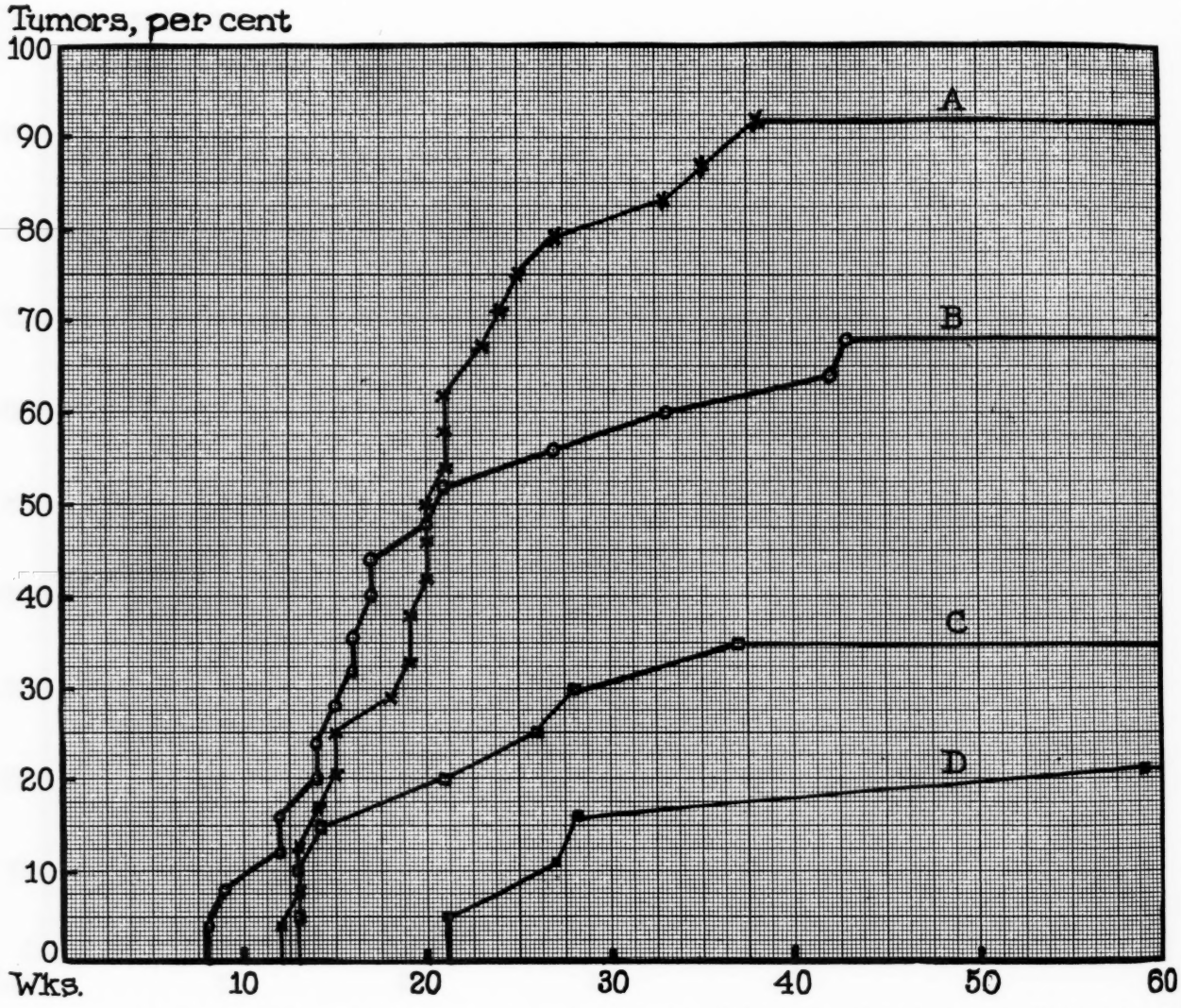

FIG. 1.—Incidence of local tumors following subcutaneous injection of 0.3 mgm. of 3,4-benzpyrene dissolved in: (A) Hydrogenated mouse fat *B* + tricaprylin; (B) Mouse fat *B* + tricaprylin; (C) Cod liver oil + tricaprylin; (D) Hydrogenated cod liver oil + tricaprylin.

No analyses of the elimination rate of benzpyrene were made in this series, mainly because the supplies of mouse fat preparations were insufficient for this purpose.

Statistical analysis.—The tumor incidence in selected pairs of experiments was tested by the χ^2 test. Where

TABLE II: INCIDENCE OF TUMORS WITH MOUSE FAT, ETC.

Solvent	No. of mice	No. died before first tumor appeared	No. with ulcers	Effectual total	Total local sarcomas			
					20 weeks		40 weeks †	
					No.	Per cent	No.	Per cent
Tricaprylin	30	8	0	22	5	23	10	46
Mouse fat *B*	30	2	0	28	10	36	17	61
Mouse fat *A* stored 3 yrs.	30	6	13	11	1	9	5	46
Original mouse fat *A* *	28	0	7	21	2	10	4	19

* Data of Dickens & Weil-Malherbe (3).

† These results were unchanged for 50th week also.

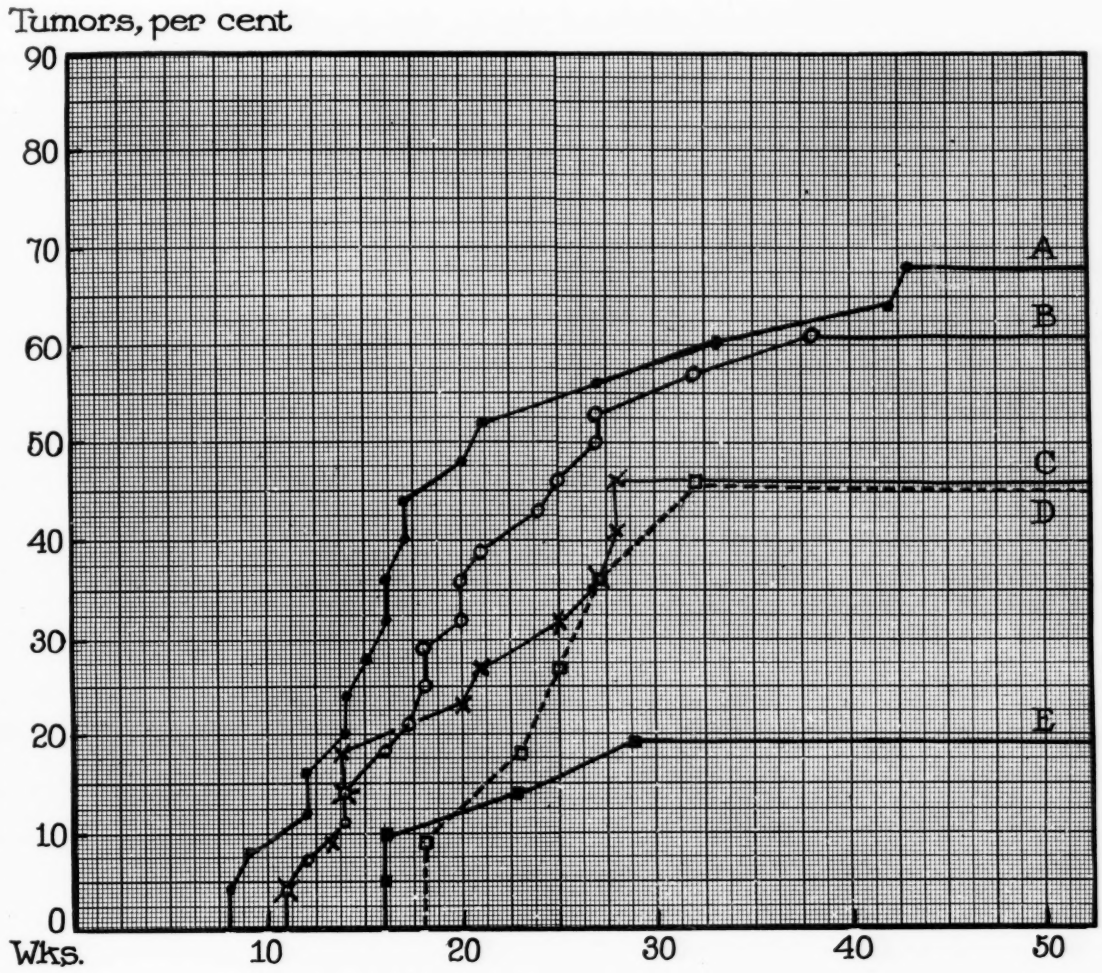

FIG. 2.—Tumor incidence with solvents: (A) Mouse fat *B* + tricaprylin; (B) Mouse fat *B;* (C) Tricaprylin, series II; (D) Mouse fat *A,* 3 years old; (E) Fresh mouse fat *A.*

one or more of the expected values were <5, Yates' correction for continuity (4) was applied. As can be seen from Table III, the difference in tumor incidence between the cod liver oil + tricaprylin and the mouse fat *B* + tricaprylin series is significant, as is the difference between natural and hydrogenated mouse fat, though only just. On the other hand, there is no significant difference between cod liver oil and hydrogenated cod liver oil. Though stale mouse fat *A* gave a seemingly much higher percentage of tumors than fresh mouse fat *A* (see Fig. 2), the difference is far from significant owing to the small effectual total in the former series caused by extensive ulceration. If the figures previously obtained with fresh mouse fat *A* (3) are compared with mouse fat *B* (undiluted) the difference is significant. Undiluted mouse fat *B* gave incidence figures that did not significantly differ from those obtained with tricaprylin. If, however, the undiluted mouse fat *B* group is combined with the mouse fat *B* + tricaprylin group, which gave very similar responses of tumor incidence (61 and 68 per cent), and if this combined group is compared with

TABLE III: X^2 TEST APPLIED TO THE TUMOR INCIDENCE CAUSED BY SUBCUTANEOUS INJECTION OF 0.3 MGM. OF 3,4-BENZPYRENE IN VARIOUS SOLVENTS

Serial No. of solvents compared *	X^2 (N = 1)	P
6/8	4.861	0.03
6/7	0.936	0.34
8/9	4.222	0.043
12/3	0.638 †	0.43
10/3	5.876	0.016
10/11	1.155	0.29
(8 + 10)/TC	5.560	0.02

* 3 = Mouse fat *A*, fresh (3).
6 = Cod liver oil + tricaprylin.
7 = Hydrogenated cod liver oil + tricaprylin.
8 = Mouse fat *B* + tricaprylin.
9 = Hydrogenated mouse fat *B* + tricaprylin.
10 = Mouse fat *B* (undiluted).
11 = Tricaprylin.
12 = Mouse fat *A*, 3 years old.
TC = Combined tricaprylin experiments (13).
† Corrected by Yates' continuity adjustment (4).

the combined results of 3 tricaprylin experiments giving final incidence figures of 33, 46 and 46 per cent (13) then the difference thus obtained by the use of the larger group becomes significant.

ELIMINATION OF BENZPYRENE

The results of the estimations of residual amounts of benzpyrene after various periods in the 4 experimental series are contained in Figs. 3 to 6. The logarithms of $S(=\mu$gm. benzpyrene found) are plotted against the time, t, in days. The straight line is the linear regression curve for the observed values. The linear regression coefficients and their standard errors are set out in Table IV.

From an inspection of Figs. 4, 5, and 6 it appears that here the premise of linearity is reasonably fulfilled and the scatter of the observations not unduly large. The position is less satisfactory in the cod liver oil series (Fig. 3), either owing to an abnormally wide

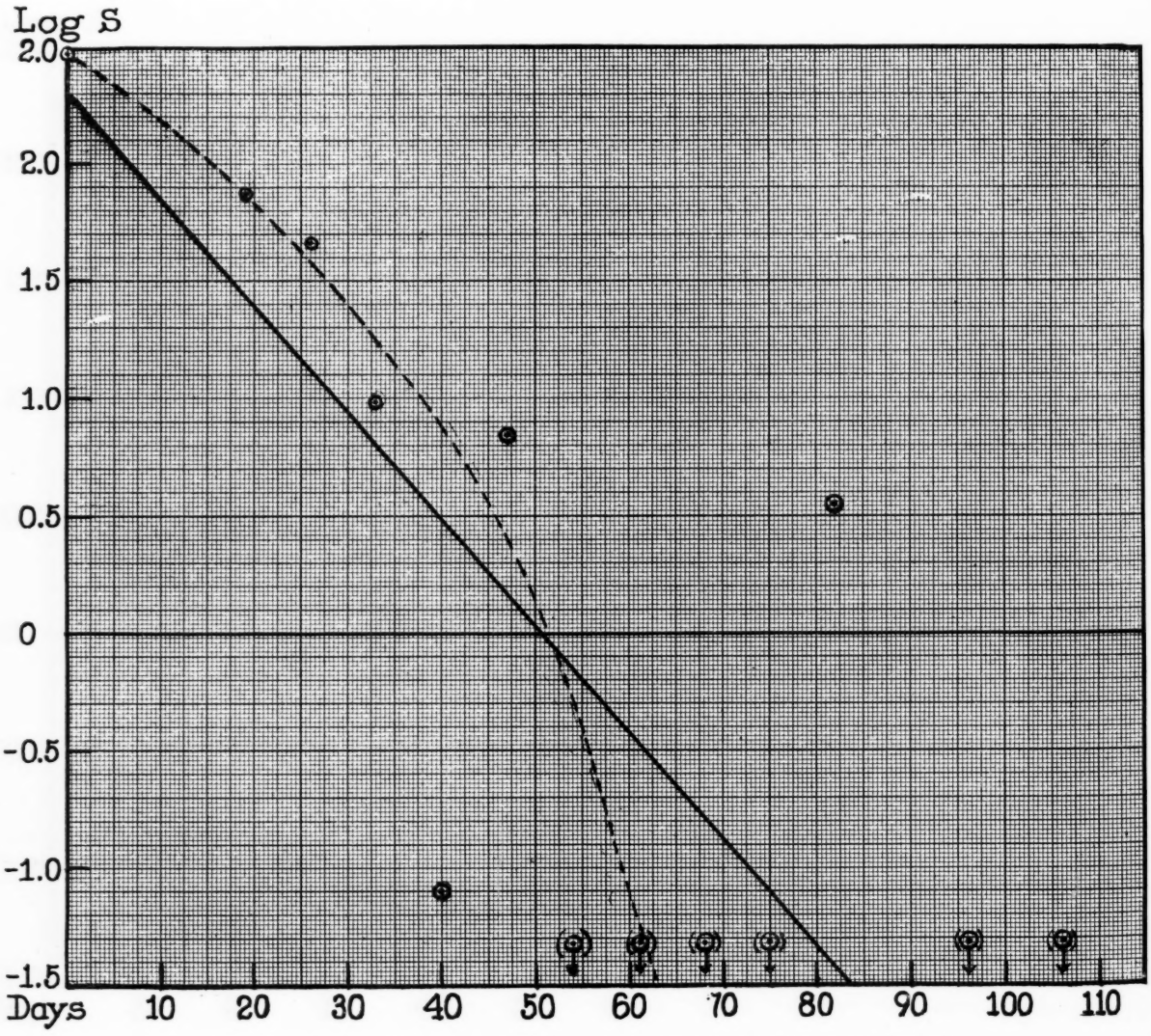

FIG. 3.—Rate of elimination of 3,4-benzpyrene after its injection in cod liver oil.

TABLE IV: RATE OF ELIMINATION OF 3,4-BENZPYRENE IN VARIOUS SOLVENTS

Serial No.	Solvent	Linear regression coefficient (b)	Standard error (S.E.)
6	Cod liver oil + tricaprylin	— 0.0439	0.0130
7	Hydrogenated cod liver oil + tricaprylin	— 0.0143	0.00462
8	Mouse fat *B* + tricaprylin	— 0.0245	0.00257
9	Hydrogenated mouse fat *B* + tricaprylin	— 0.0130	0.00310

scattering or to a striking digression from linearity. If the latter assumption is correct, the rate of elimination might perhaps follow a course indicated by the dotted line. The bracketed points in this graph represent analyses where no measurable amounts of benzpyrene could be detected; *i. e.,* less than about 0.5 μgm. In conformity with the standard previously adopted (12), of the analyses yielding no measurable amounts of benzpyrene those that occurred after the last measurable observation were omitted from the statistical evaluation. This applied here to the last two points of the series, corresponding to 96 and 106 days. For the other bracketed points values of 0.05 μgm. were arbitrarily assumed, as their omission would falsify the result, but it should be remembered that the true position of these points would, in reality, lie in a negative direction along the y-axis. A certain amount of bias is introduced by the arbitrary values, which tends to decrease the regression coefficient below its true value.

Statistical analysis.—The probability accruing from the difference of paired regression coefficients (b_1, b_2) was calculated by the formula:

$$t_{(n_1+n_2-4)} = \frac{b_1 - b_2}{\sqrt{(\text{S. E.}_{b_1})^2 + (\text{S. E.}_{b_2})^2}}$$

The results are shown in Table V. Hydrogenation of both mouse fat *B* and cod liver oil causes a significant retardation of elimination. The elimination rate is almost identical in the two hydrogenated solvents. Although the higher elimination rate with cod liver oil is not significantly different from that obtained with mouse fat *B,* there is at least a suggestion that a significant difference might have been revealed by more extensive data, especially in view of the pro-

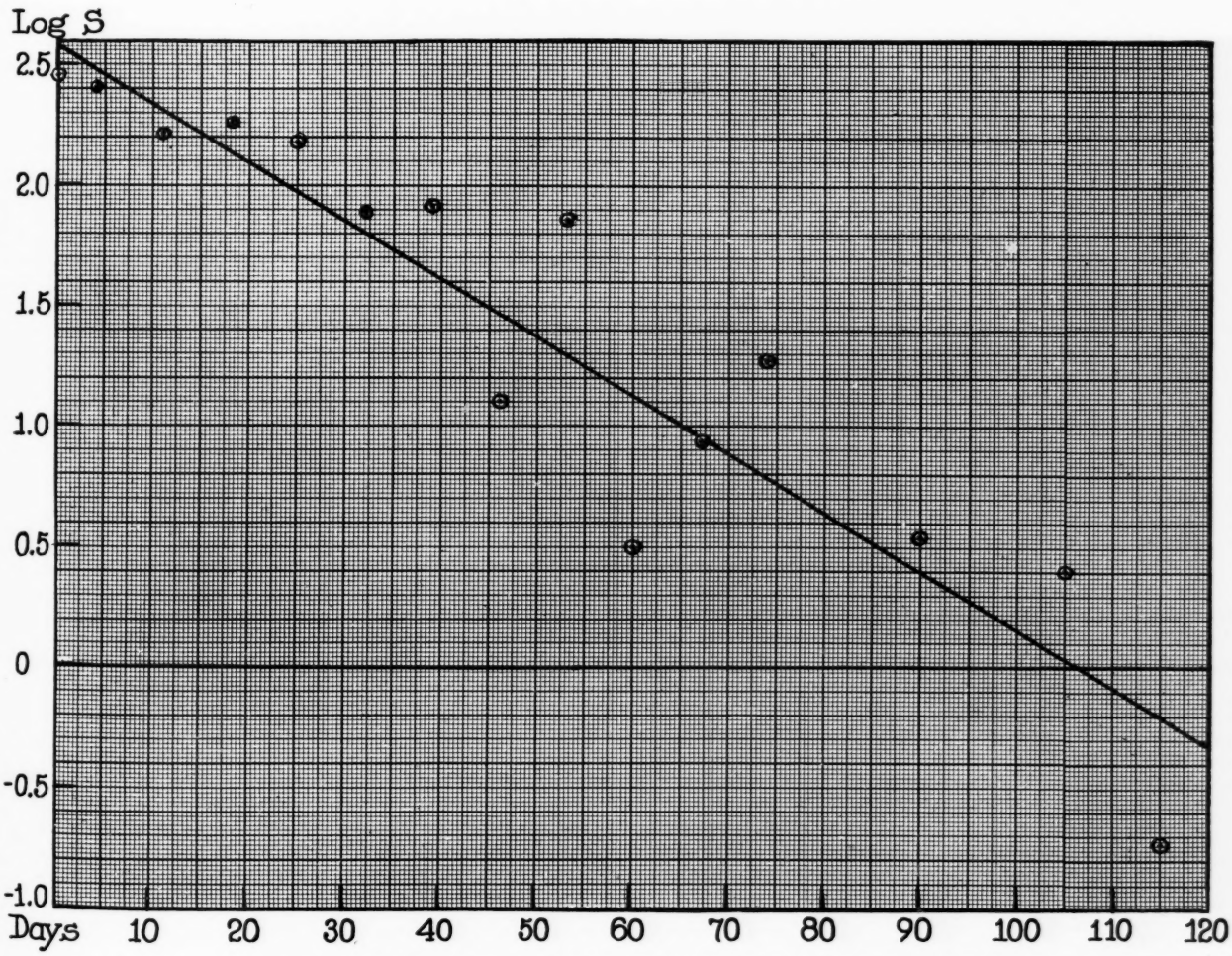

FIG. 4.—Rate of elimination of 3,4-benzpyrene after its injection in mouse fat *B.*

nounced scatter and of the bias due to the introduction of arbitrary values in the cod liver oil series.

DISCUSSION

In the experiments with hydrogenated solvents described in the present paper, as in those with purified phospholipins (12), the solid nature of these substances made it necessary to soften them by the admixture of an equal amount of tricapylin. The unhydrogenated fats were similarly diluted with an equal amount of tricaprylin; in addition, results are available for the effects of the same solvents when used undiluted. A comparison of the two sets of experiments shows that the incidence of tumors after 40 weeks was 37 per cent

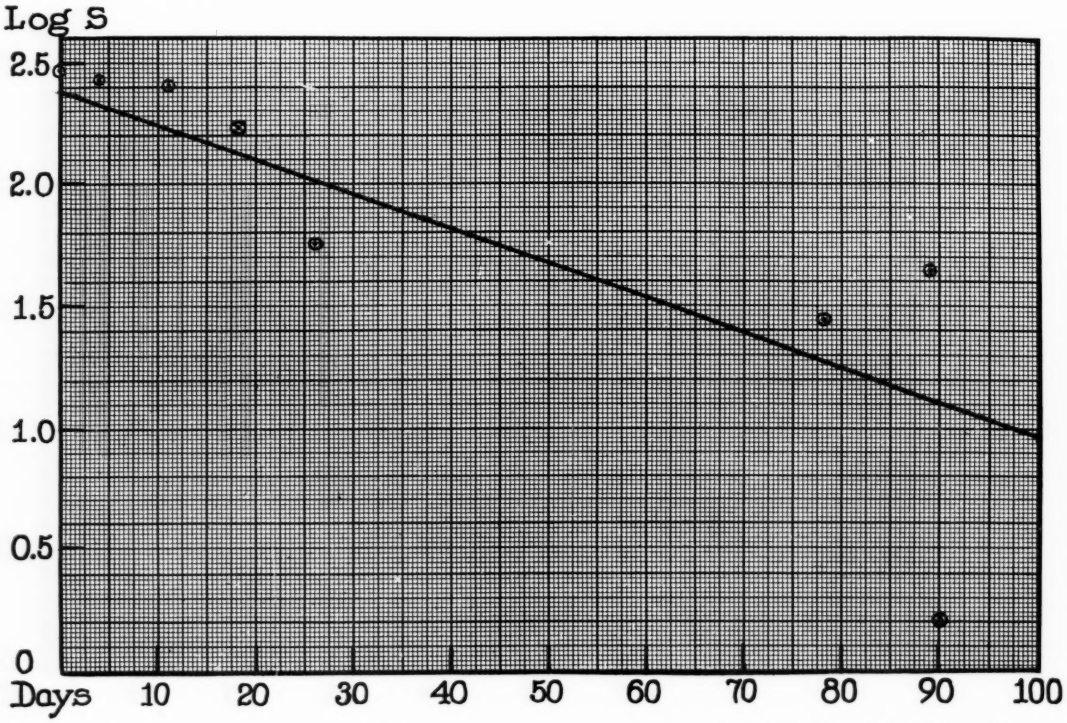

FIG. 5.—Rate of elimination of 3,4-benzpyrene after its injection in hydrogenated cod liver oil.

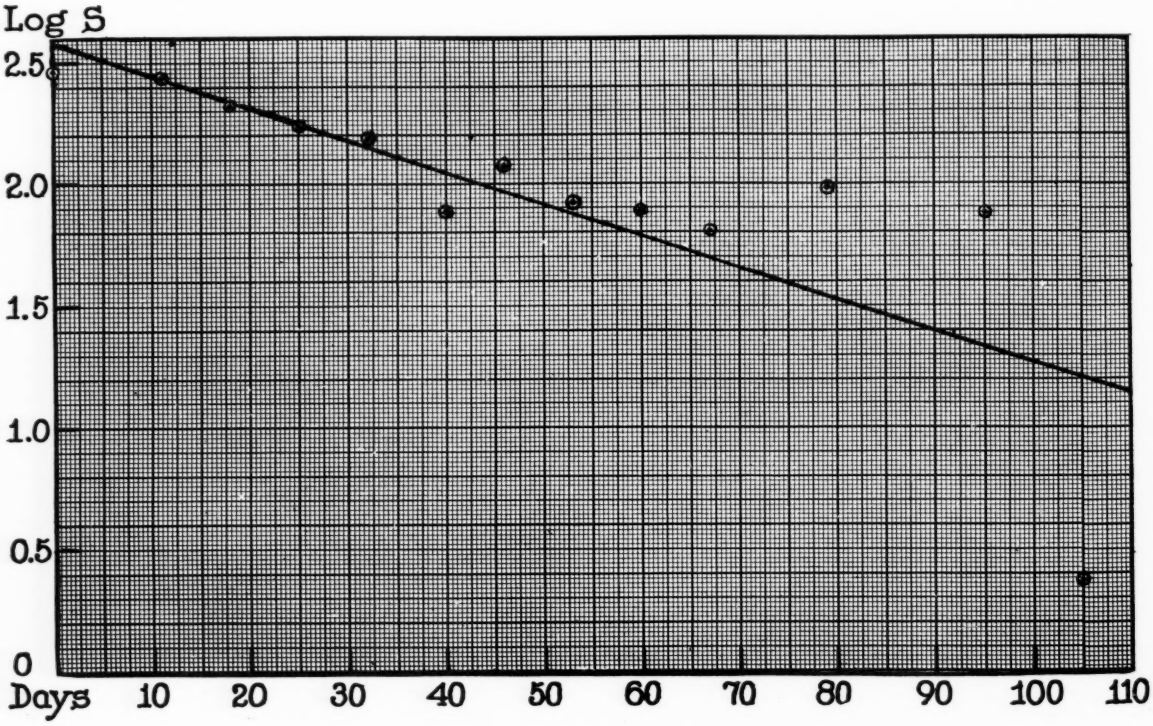

FIG. 6.—Rate of elimination of 3,4-benzpyrene after its injection in hydrogenated mouse fat *B*.

TABLE V: SIGNIFICANCE OF DIFFERENCES OF RATE OF ELIMINATION OF 3,4-BENZPRENE IN VARIOUS SOLVENTS

Serial No. of solvents compared *	No. of observations	t	P
6/7	19	2.138 days	0.05
6/8	26	1.459	0.1 — 0.2
6/9	24	2.304	0.03
7/8	23	1.942	0.06
8/9	28	2.873	< 0.01
7/9	21	0.231	0.8

* See footnote to Table III.

for cod liver oil and 61 per cent for mouse fat *B*, and for the same solvents diluted with an equal amount of tricaprylin 35 and 60 per cent. This dilution did not, therefore, obscure in any way the effects of the undiluted solvents, and tricaprylin may be regarded as a truly neutral diluent. The behavior of more dilute solutions of phospholipins in tricaprylin and the effect of such dilution on carcinogenesis and elimination will be considered in the subsequent paper (13).

The preparation of mouse fat *B* used for the present investigation proved to be entirely devoid of inhibitory properties, in contrast to the sample of mouse fat *A*, used 3 years ago (3). Mouse fat *B* could even be shown to possess some cocarcinogenic activity compared with tricaprylin. The difference between the two samples of mouse fat is probably connected with the fact that mouse fat *A* was prepared from whole mice, including the lipid-rich tissues such as brain and liver. Even though acetone was used in the preparation of the extract, its phospholipin content was 1.5 per cent. At the time of these first experiments it seemed rather improbable that such a low concentration of phospholipin would much influence carcinogenesis, but we now have evidence that amounts of phospholipins of this order may have an inhibitory action (13). In mouse fat *B* the concentration of phospholipins was only about one-thirtieth of that in mouse fat *A*, and it may well be that this fact is associated with the difference of the carcinogenic activity of benzpyrene in these two solvents.

When mouse fat *A*, after three years' storage, was tested again the tumor incidence was rather higher than in the first experiment (3) and now reached the level of tricaprylin. Although, owing to the small effective total of animals in this group, a change caused by the storage cannot be demonstrated statistically at present, the results provide at least a suggestion that such a change may in fact have occurred. This would agree well with the theory that the inhibitory effect of mouse fat *A* was due to its phospholipin content, as phospholipins would undergo oxidation in air and might thus lose their protective properties.

The effect of hydrogenation.—If the effect of hydrogenation on the elimination of benzpyrene is considered first, it appears that the rate of elimination is significantly decreased, whether the solvent is cod liver oil or mouse fat *B*. The rate of elimination is practically the same in the two hydrogenated solvents, and also in pure tricaprylin (13). The tumor incidence, on the other hand, is not greatly affected by hydrogenation; with cod liver oil it is slightly depressed by hydrogenation, but this effect does not reach the level of statistical significance; with mouse fat *B* hydrogenation significantly increases the tumor incidence. Hydrogenation thus has the effect of accentuating still further the already significant difference in the tumor response to the natural solvents.

The *prima facie* conclusion from these facts would be that there is no correlation between elimination rate and carcinogenesis. While this is apparently true for the experiments here discussed, it should not be overlooked that hydrogenation introduces a complication, in that it greatly alters the physical nature of the solvent. The retardation of the elimination of benzpyrene after hydrogenation may be due mainly to these physical factors, which entailed a slowing up of the diffusion within the solid pellet of solvent. The cocarcinogenic or anticarcinogenic properties of the solvents, however, may have remained unaffected or even been enhanced by the change in the physical state of the solvent. If this interpretation is correct, a correlation between elimination rate and carcinogenesis could be expected only between solvents of a similar physical nature. On the other hand, it should be pointed out that the elimination rate with the hydrogenated fats is not slower than with the oily tricaprylin (13), which might be considered as evidence against any considerable effect of the physical factor.

Our results differ from those of Leiter and Shear (8), who found in experiments made under somewhat different conditions that triglycerides containing saturated fatty acids of high molecular weight, such as tristearin or tripalmitin, exerted an inhibitory influence on tumor formation. Our samples of hydrogenated fats must have consisted largely of these triglycerides, yet with the hydrogenated mouse fat, at any rate, there was no indication of inhibition, but, on the contrary, a significant promotion. Nor do we think that the presence of saturated glycerides in our inhibitory sample of mouse fat *A* could account for its anticarcinogenic properties. In our first publication (3) we separately tested liquid mouse fat and the solid portion that separated from it at room temperature, with the result that the solid was not more inhibitory than the liquid fraction.

Cod liver oil, like other compounds containing long-chain fatty acids with two or more unconjugated double bonds, produces a unique type of tissue reaction after subcutaneous injection (5). The subcutaneous

deposit is converted into a semisolid mass, insoluble in the usual fat solvents, and with pronounced affinity for carbolfuchsin. This material first appears at the interphase with the regional tissues and gradually extends, forming a system of membranes, globules, and granules (5, 6). We have regularly observed this reaction with solutions of benzpyrene in cod liver oil. The unusual features observed with cod liver oil as solvent; *i. e.*, the wide scatter found in the elimination analyses, and the low tumor incidence combined with a shortening of the latent period and an increased rate of elimination of benzpyrene (13), may well be connected with this exceptional tissue reaction.

Finally, attention should be drawn to the fact that an increased rate of elimination as in the natural mouse fat *B* and cod liver oil experiments was associated with a shortening of the latent period as compared with the hydrogenated fats and tricaprylin. This point will be more fully discussed in a following paper.

Summing up, we arrive at the following conclusions: (a) neither highly unsaturated nor fully saturated fatty acids present as triglycerides in mouse fat can be held responsible for its inhibitory action on benzpyrene carcinogenesis; (b) the rate of elimination of benzpyrene is more rapid with the unsaturated fats (cod liver oil and mouse fat) than with the same fats after hydrogenation, but this phenomenon is so much complicated by the different physical state before and after hydrogenation that its correlation with carcinogenesis was not to be expected, and no such correlation was in fact found; (c) there is, however, a tendency for the presence of unsaturated fatty acids to diminish the latent period.

SUMMARY

Four groups of mice were given subcutaneous injections of a single dose of 3,4-benzpyrene dissolved in 50 per cent solutions in tricaprylin of the following: cod liver oil, hydrogenated cod liver oil, mouse fat, hydrogenated mouse fat. The incidence of tumors with cod liver oil was about the same as that found for tricaprylin, which we adopt as standard solvent. There was a significantly higher incidence in the mouse fat series, whether hydrogenated or natural solvent was used, than in the two cod liver oil solvents. Hydrogenation had a tendency to decrease the tumor incidence in the cod liver oil series, but the difference was not significant. In the mouse fat series, hydrogenation caused a significantly increased incidence. The latent period tended to be shorter with unsaturated fats.

The rate of elimination was determined for these solvents. It was accelerated in the unsaturated solvents, but after their hydrogenation the rate of elimination was slowed down to that observed with tricaprylin.

The fact that the sample of mouse fat used in these experiments was not anticarcinogenic was confirmed by repeating the test with the same sample undiluted with tricaprylin. Tricaprylin behaved as an entirely neutral diluent. The lack of inhibitory action of the present sample of mouse fat is attributed mainly to its much lower content of phospholipins than the sample previously used by us.

From consideration of these results we conclude that: (a) The anticarcinogenic action observed by others as well as ourselves in certain samples of mouse fat is due neither to their content of highly unsaturated fatty acids (present as glycerides) nor to the presence of fully saturated ones. (b) The rate of elimination of benzpyrene in these experiments bore no direct relationship to the cocarcinogenic or anticarcinogenic nature of the solvent, but it is pointed out that this may be due to complication by physical factors. One consistently observed correlation was the shortening of the latent period accompanying rapid elimination.

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Factors Affecting Carcinogenesis

IV. The Effect of Tricaprylin Solutions of Cholesterol and Phospholipins

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In this series of publications (5, 6, 22) we have studied the incidence of local sarcomas resulting from the subcutaneous injection of a standard dose of 3,4-benzpyrene into mice. It was found (6) that there was a significantly higher tumor incidence when the solvent was a sample of mouse fat consisting almost entirely of neutral glycerides than with another sample of mouse fat (5) that was less pure and contained appreciable amounts of other lipids, notably cholesterol and phospholipins. It was of interest to study the effect of these two lipids independently in a synthetic medium (tricaprylin) at about the concentration in which they were present in the sample of mouse fat that inhibited carcinogenesis. We had previously found (22) a strong inhibitory effect of phospholipins, but as the concentrations then employed were very much higher than that in the mouse fat, these experiments did not prove that the inhibitory effect of mouse fat was due to its phospholipin content. Furthermore, in the light of more recent experience we feel that two objections may be raised against the experiments: (a) our mixtures of equal parts of lecithin or cephalin with tricaprylin were plastic pastes, the consistency of which differed greatly from that of the oily solvents with which they were compared. We now hold that, to exclude physical factors, the consistency of the solvents should be similar. (b) The implantation technic by which the lipid mixtures were applied in most cases does not give a sufficient guarantee against primary leakage of the soft material through the incision; and the slough that always forms on the wound may conceal a secondary leakage through ulceration.[1] In view of these facts a reinvestigation of the phospholipin effect seemed indicated.

EXPERIMENTAL

Preparation of Phospholipins

Two sheeps' brains (280 gm.) were extracted 3 times at 0° C. for 24 hours with acetone, 1 litre for each extraction. The dry acetone-insoluble residue (55 gm.) was shaken at room temperature under nitrogen in 4 changes of ethanol, 800 ml. The alcohol-insoluble residue was twice extracted with light petroleum at room temperature. The alcohol and petroleum solutions were evaporated (all distillations were done in nitrogen under reduced pressure), the combined residues were taken up in peroxide-free ethyl ether (300 ml.), and 1 litre of acetone was added. This acetone precipitation was repeated with the solution of the first precipitate in ether, and the resulting precipitate was dissolved in chloroform, washed with dilute sodium chloride solution, and the dried chloroform layer evaporated. The ether solution of the residue was cleared by centrifuging and then precipitated with acetone. This precipitation was repeated 5 times, until the material gave a perfectly clear ethereal solution. The final acetone precipitate was extracted with ethanol and the filtered solution evaporated, dissolved in ether, and precipitated with acetone. The acetone precipitate was dissolved in ether and left overnight at 0° C. It was then centrifuged and again precipitated with acetone. After solution in ethanol, the lecithin was finally reprecipitated from ether by acetone: the yield was about 1 gm. of dry substance.

The greater part of the material remained in the alcohol-insoluble fraction. This was dissolved in ether and the clear solution precipitated with acetone. The precipitated cephalin fraction was thoroughly washed with acetone and dried.

[1] We had originally hoped to supplement our previous evidence by the following experiment: a large batch of mouse fat was prepared from the whole carcasses of mice by a method similar to that used for the preparation of our first sample of mouse fat, mouse fat *A*, (5). Part of it was to be freed from phospholipins and another part from cholesterol; the two purified fractions were then to be compared with the original fat as solvents for benzpyrene. Unfortunately this plan was frustrated by the fact that this sample of mouse fat caused very severe ulceration when used as a solvent for the subcutaneous injection of benzpyrene. This property persisted even after it had been practically freed from phospholipins by repeated acetone precipitations and after further purification by repeated treatment with charcoal and extraction with acid.

Preparation of Mixed Phospholipins for Injection Experiments

Portions of 1 gm. each of the cephalin and lecithin preparations were mixed, dissolved in ether, and the solution, cleared by centrifuging, was precipitated with acetone. The dried precipitate contained 3.96 per cent of phosphorus. Sufficient of the mixed phospholipin to give a 3 per cent solution was dissolved in a solution of 3,4-benzpyrene in tricaprylin containing 1 mgm./ml. of the hydrocarbon. As in all these experiments, each mouse received 0.3 ml. of the solution; *i. e.*, 0.3 mgm. of benzpyrene.

The control solution contained the same quantity of benzpyrene in pure tricaprylin. The 3 per cent solution of cholesterol in tricaprylin used also had the same benzpyrene content.

Animal Experiments

The technic of injection has already been fully described (6). The only difference in the present series was the use of pure strain mice (males of the Glaxo FF strain). For each of the 3 solvents a group of 45 animals was injected; 30 were used for the observation of tumor incidence and the remainder for estimation of the rate of elimination of benzpyrene; again the details were as previously described (6).

Ulceration after the injection was negligible with the pure tricaprylin and the cholesterol solutions, but it was found on careful observation to be present at about the tenth day in one-third of the phospholipin series; all such mice were, of course, discarded. This unfortunately reduced the effectual total in the phospholipin series to about 20, and circumstances did not allow their replacement by similar mice. No late ulceration or sloughing occurred in any of these experiments.

The mice used for analysis were also closely observed for possible ulceration, and only those free of it were taken for benzpyrene estimations.

Observations During Latent Period

Soft subcutaneous lumps or cysts were palpable for periods varying from a few weeks to the whole duration of the experiment (30 weeks) in almost all the mice injected. The usual course was for the size to diminish slowly until they were no longer palpable or until a local sarcoma began to appear, but in some mice no diminution could be felt. In the ulcerated mice (all of which were discarded) the lumps promptly vanished after the appearance of the ulcer. There was little difference between the tricaprylin and cholesterol series, but in the phosphatide series the lumps tended to be larger and more persistent; however, occasional large and persistent lumps were seen in the cholesterol series also.

Tumor Incidence

The results are summarized in Table I, which gives the numbers and percentage incidence of tumors after 20 and 30 weeks, and in Fig. 1. The incidence with tricaprylin as solvent is in good agreement with our other experiments in which we tested this solvent (22, 6), the actual figure being 46 per cent in the present series. The first sarcoma appeared after 11 weeks, and the median incidence was at 20 weeks. In 1 animal killed owing to the presence of an ulcer after 10 days, the bile and intestines showed brilliant fluorescence, presumably due to metabolism of the injected benzpyrene. No distant tumors were observed in any of the mice injected with these 3 solvents; all the neoplasms were local sarcomas.

Table I: Tumor Incidence

Solvent	No. of mice	No. died before first tumor	No. with ulcers	Effectual total	Total local sarcomas			
					20 weeks		30 weeks	
					No.	%	No.	%
Phosphatides (3%) in tricaprylin	30	2	11	17	2	12	8	47
Tricaprylin	30	2	2	26	10	38	12	46
Cholesterol (3%) in tricaprylin	30	1	1	28	22	79	23	82

In the cholesterol series the total incidence was high: 82 per cent. Two tumors appeared after 10 weeks, and the median time was 15 weeks. Animals that died 10 and 16 weeks after injection still had a local oil depot.

In the phosphatide series the main effect was a delay in tumor incidence, as compared with tricaprylin. The first tumor arose after 12 weeks, and the median incidence was at 24 weeks. Up to the 23rd week there were only 2 sarcomas in an effectual total of 17 mice, whereas at the same period in the tricaprylin series the total was 11 out of 26 mice. The difference from the cholesterol series was even more pronounced; the latter had at this period 22 sarcomas in 28 mice.

Statistical Analysis

The significance of the differences in tumor incidence according to the solvent used were analyzed by the χ^2 test. The solutions containing cholesterol and

phospholipins respectively were compared with the pure tricaprylin solvent as the standard of reference for two periods; *i. e.*, after 20 and after 30 weeks. The results are shown in Table II. In the comparison between the phospholipin solution and tricaprylin after 20 weeks one of the "expected" values was below 5 (4.75); Yates' continuity adjustment was therefore applied in this case (9). A *P*-value of 0.13 was found.

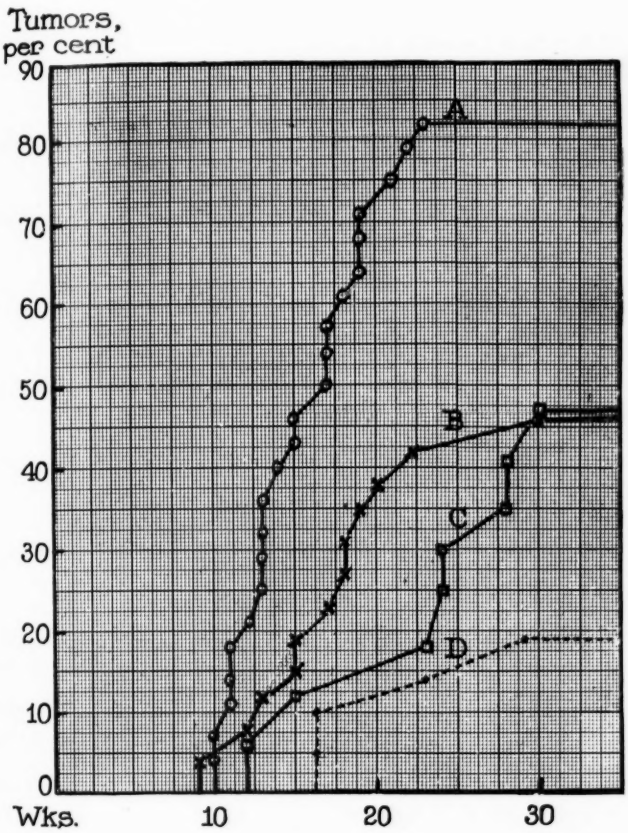

FIG. 1.—Incidence of local tumors following subcutaneous injection of 0.3 mgm. 3,4-benzpyrene dissolved in: (A) cholesterol, 3 per cent solution in tricaprylin; (B) tricaprylin (series III); (C) lecithin, 1.5 per cent; and cephalin, 1.5 per cent in tricaprylin. Curve (D) shows the behavior of the original sample of mouse fat (5).

Without correction for continuity $P=0.06$. The true value of P lies between these two values, near 0.1. This is not a significant result in the statistical sense, but it suggests that more extensive data might satisfy the criterion of significance. After 30 weeks the percentage incidence in the phospholipin series had risen to that in the tricaprylin series. These two groups were therefore combined for the comparison with the cholesterol series. The higher tumor incidence in the latter is highly significant, whether compared with the tricaprylin group after 20 weeks or with the combined tricaprylin and phospholipin groups after 30 weeks.

We now have available the results of three experiments with tricaprylin as solvent. In addition to the present experiment the first was described in Part II (22), and the second in Part III (6) of this series of publications. A combination of these results, which are in reasonable agreement, provides sufficient material for a standard of reference by comparison with which it is possible to distinguish between cocarcinogenic and anticarcinogenic activity. Table II includes some data taken from our earlier papers, which are now compared statistically with the combined tricaprylin results. The original sample of mouse fat (mouse fat *A*, fresh), which we have so far regarded

TABLE II: χ^2 TEST APPLIED TO FIGURES OF TUMOR INCIDENCE

Serial No. of experiments compared *	χ^2 (N = 1)	*P*
	AFTER 20 WEEKS	
13/14	3.641	0.06
	2.435 †	0.13
15/14	7.361	< 0.01
	AFTER 30 WEEKS	
(13 + 14)/15	9.014	< 0.01
3/TC	2.037	0.16
1/TC	13.97	< 0.01
(1 + 2)/TC	10.67	< 0.01

* 1 = sesame oil (5).
2 = arachis oil (5).
3 = mouse fat *A*, fresh (5).
13 = tricaprylin containing 3% phospholipins.
14 = tricaprylin.
15 = tricaprylin containing 3% cholesterol.
TC = combined tricaprylin experiments.

† Corrected by Yates' continuity adjustment.

as anticarcinogenic, is shown not to differ significantly from tricaprylin, although, as in the case of the present phospholipin experiment, the fairly low value of $P=0.16$ suggests a tendency towards inhibition. On the other hand, the results of the sesame oil experiment and the combined results of the sesame and arachis oil experiments reveal a highly significant cocarcinogenic activity of these solvents. It has previously been shown that our sample of mouse fat *B* possessed similar properties (6).

ELIMINATION OF 3,4-BENZPYRENE

Figs. 2 to 4 represent for each of the three series the results of benzpyrene analyses performed with mice selected at random and killed at regular intervals of 1 week. In the Figures, log S (= log of quantity of benzpyrene in micrograms remaining) is plotted against the number of days. The straight line is the linear regression curve. The numerical values of the linear regression coefficients and their standard errors are

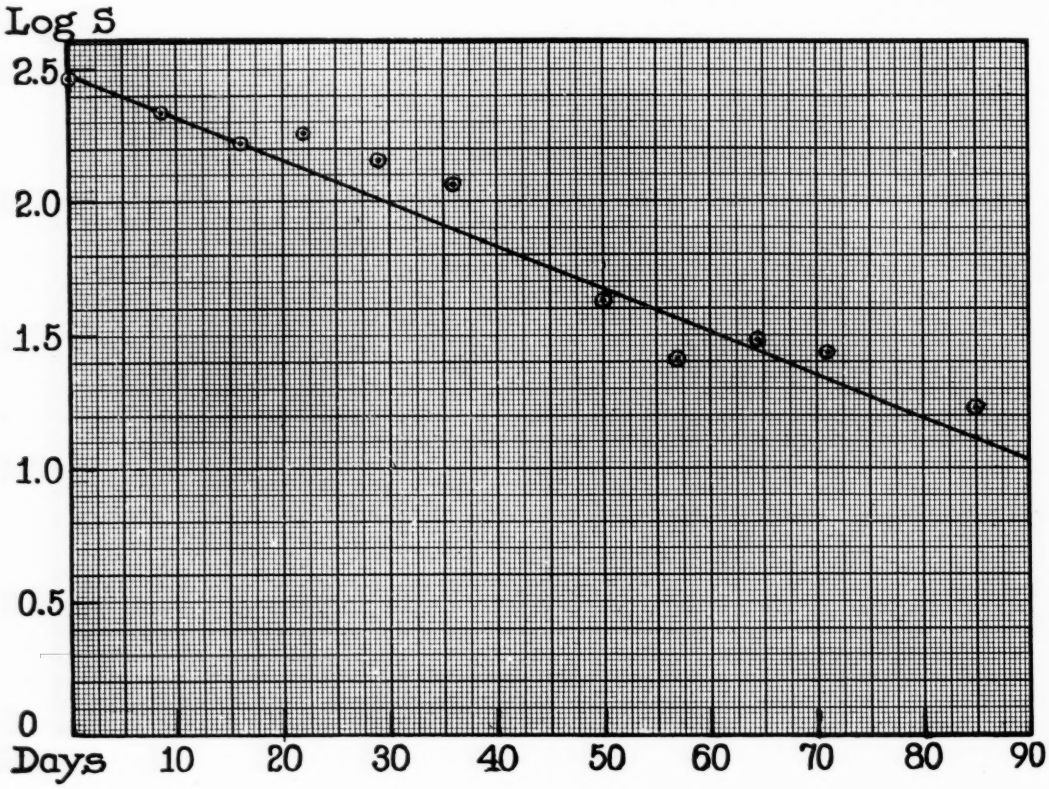

FIG. 2.—Rate of elimination of 3,4-benzpyrene after its subcutaneous injection in tricaprylin.

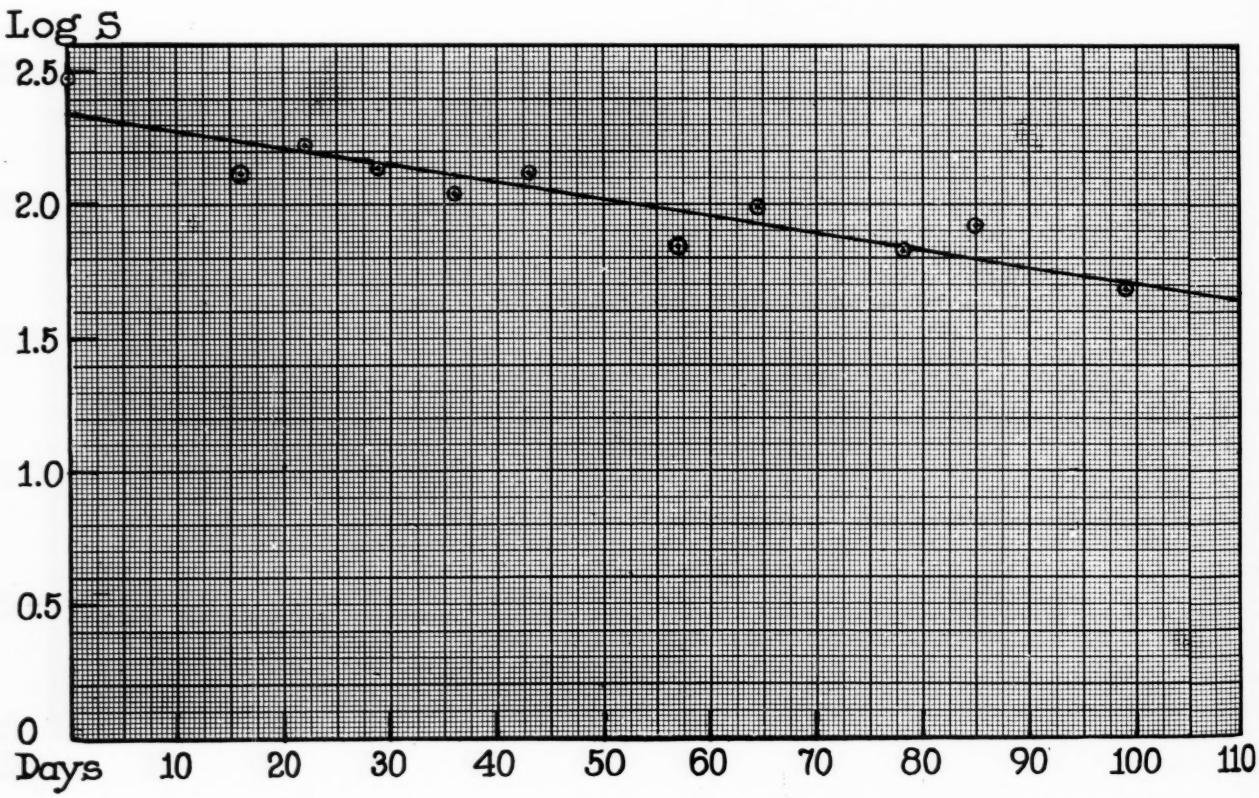

FIG. 3.—Rate of elimination of 3,4-benzpyrene after its subcutaneous injection in tricaprylin containing phospholipins.

TABLE III: ELIMINATION OF BENZPYRENE WITH VARIOUS SOLVENTS

Serial No.	Solvent	No. of observations	Linear regression coefficient, b	Standard error of b (S.E.)
13	Phospholipins (3%) in tricaprylin	11	— 0.00619	0.00106
14	Tricaprylin	11	— 0.0157	0.00116
15	Cholesterol (3%) in tricaprylin	12	— 0.0274	0.00354

with a wider scatter, an observation similar to that made with cod liver oil (6). If the individual elimination constants [2] in the cholesterol series are considered, they are seen to fall roughly into two classes: values between 0.03 and 0.04 and values around 0.015. The latter value corresponds to the excretion rate observed with pure tricaprylin, so that it seems as if not all the animals would respond to the admixture of cholesterol in tricaprylin.

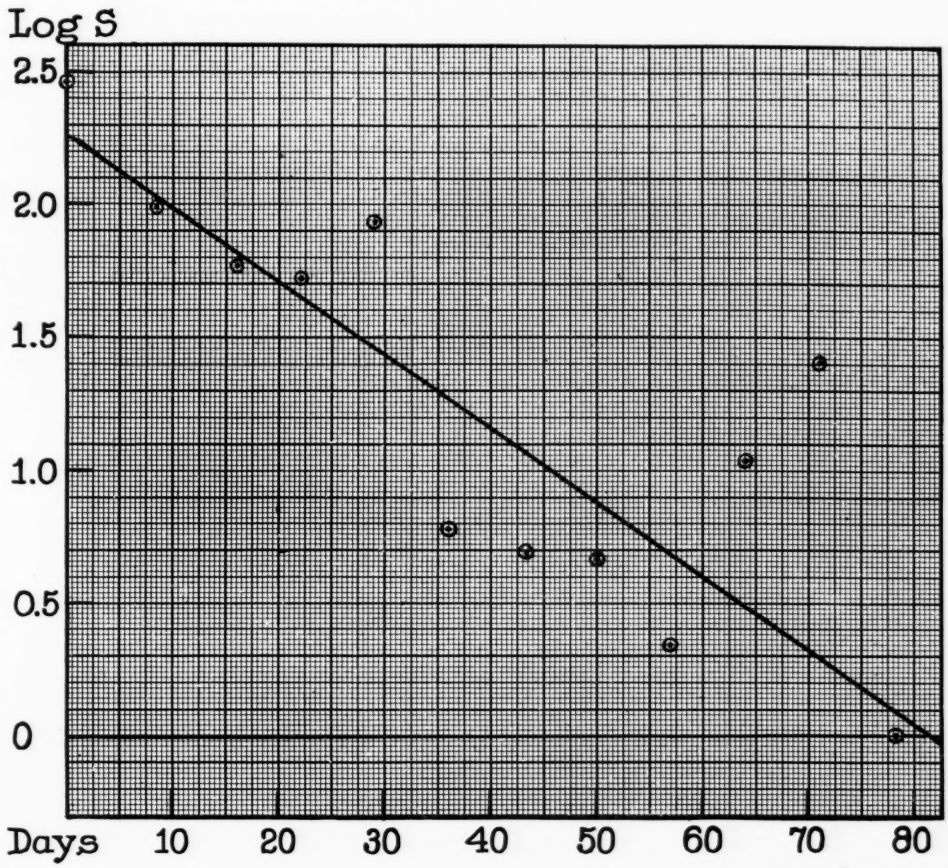

FIG. 4.—Rate of elimination of 3,4-benzpyrene after its subcutaneous injection in tricaprylin containing cholesterol.

contained in Table III, and the probabilities corresponding to the differences between pairs of regression coefficients in Table IV. The elimination is fastest in the cholesterol solvent experiment, intermediate in the tricaprylin experiment, and slowest in the phospholipin experiment. All the differences are highly significant. In none of the experiments is there any obvious digression from linearity, although the faster excretion in the cholesterol series was also associated

TABLE IV: STATISTICAL ANALYSIS OF ELIMINATION RESULTS

Serial No. of solvents compared (see Table II)	t	P
15/14	3.125	< 0.01
13/14	6.060	< 0.01

DISCUSSION

In considering cocarcinogenic or anticarcinogenic effects it is important to have a stable and reproducible standard of reference, which should itself be absolutely neutral towards carcinogenesis. Tricaprylin, which we chose following the example of other authors (12, 18), seems to fulfill these requirements adequately. The results of the three independent experiments reported in this paper and in Parts II and III of the series (6, 22) are in reasonably close agreement, in spite of the fact that they were carried out on different mouse

[2] $k = \frac{1}{t} \log \frac{300}{S}$ (see 21).
The linear regression coefficient, b, is the statistical mean value of k.

populations. The somewhat lower tumor incidence in the first experiment, 33 per cent (22), may have been due to the fact that old female mice of mixed stock were employed, for Leiter and Shear found the incidence of induced tumors to be 44 per cent higher in the males than in the females of strain A (12). The second and third experiments gave identical figures of percentage incidence (46 per cent), although the second was done on male mice of mixed stock and the third, reported in the present paper, on male mice of an inbred strain. Another important requirement that is satisfied in our experiments is that the carcinogenic dose should be so chosen that the tumor response in the experiment that is to serve as the standard amounts to about 50 per cent; in that case even weak cocarcinogenic or anticarcinogenic influences have an equal chance of shifting the result.

By comparison with tricaprylin the solvents so far tested in our experiments may be arranged as follows: (a) a group of cocarcinogenic solvents comprising sesame and arachis oil, mouse fat *B*, and tricaprylin containing 3 per cent cholesterol; (b) a group probably possessing anticarcinogenic properties; *i. e.*, mouse fat *A*, and tricaprylin containing 3 per cent phospholipins; (c) cod liver oil, with a tumor incidence resembling that of tricaprylin itself.

In the cocarcinogenic group we find a statistically highly significant increase of tumor incidence that seems to be associated with a shortening of the latent period. We also find, at any rate in the mouse fat *B* and the cholesterol-tricaprylin group, a significant increase of the elimination rate of benzpyrene. Statistical comparison between the linear regression coefficients for the elimination rate shows that the coefficient for mouse fat *B*, as determined in the preceding paper (6), is significantly higher than that found for pure tricaprylin, as reported in the present investigation ($t_{(N=22)}=3.126$; $P=<0.01$).

It is possible, though it cannot be proved at present, that the cocarcinogenic effects are due entirely to the sterol content of these solvents. This would of course presuppose that sitosterol, which occurs in oils of plant origin in considerable amounts, is in this respect equivalent to cholesterol. It is intended to test this point by experiment. The cholesterol content of mouse fat *B* (0.167 per cent) was low, but not negligible; but its cocarcinogenic effect was also lower than that of the other members of the group. An interesting cocarcinogenic effect of cholesterol has been described by Baumann, Rusch, Kline, and Jacobi (1, 17), who found that tumors induced by benzpyrene painting or by ultraviolet irradiation of mouse skin were stimulated by the application of cholesterol in an oily medium (cottonseed oil), but not by cholesterol in benzene.

In the group termed anticarcinogenic the effects are less striking. Though the percentage incidence of tumors with mouse fat *A* was lower than the combined results of the three tricaprylin experiments, the difference is not statistically significant. With phospholipins in tricaprylin the final tumor incidence reaches the level of that observed with tricaprylin, but there was a pronounced lengthening of the latent period. The impressive nature of the contrast between vegetable oil and mouse fat in our original experiment (5) was of course due to the fact that the comparison was unwittingly made with solvents that we have now reason to believe are strongly cocarcinogenic. Nevertheless, we still hold that there are solvents with anticarcinogenic properties, and that phospholipins are at least partly responsible for their action. Though our evidence may not yet be statistically convincing, it is reinforced by the following facts: (a) the association of accelerated elimination with shortened latent period observed in the cocarcinogenic group has its complete counterpart in the experiment with phospholipins in tricaprylin, where a lengthening of the latent period was accompanied by a much delayed excretion. (b) Though our older experiments, in which pellets of ox brain lipids (5) and of purified lecithin and cephalin (21) were implanted, are not up to the stringent standards we have now adopted, owing to the different physical state of the solvents and the possibility of unobserved leakage through the incision wound, they point at least in the same direction. (c) There can be little doubt that the crude extracts of "egg yolk fat" or "chicken fat" (15), of unpurified mouse fat (14, 16), and rat fat (20), by the use of which the inhibitory action of solvents was first observed, contained considerable amounts of phospholipins, in view of the fact that they were prepared from phospholipin-rich sources without purification. Some of them (14) were probably more powerful inhibitors than our preparation of mouse fat *A*, which had been partially purified by acetone precipitation. It seems also quite probable that the puzzling variability of different batches of lard as a solvent (12) resides in the very variable phospholipin content of this material (13).

The case of cod liver oil is rather complicated. Here we have a rapid elimination combined with a "neutral" tumor incidence. Cod liver oil contains from 0.5 to 2.0 per cent cholesterol (11), but it also contains an assortment of unusual lipids, sterols, steroids, hydrocarbons, and antioxidants, any one of which may exert an antagonistic effect on cholesterol. Furthermore, cod liver oil causes a peculiar tissue reaction (6).[3] The possible play and counterplay of a variety

[3] A study of the tissue reaction to cocarcinogenic and anticarcinogenic solvents would be of some interest, but our pre-

of factors precludes a simple explanation. The same is true for lanolin, which, though a rich source of cholesteryl esters, prevents carcinogenesis in the skin after methylcholanthrene painting (19). In order to explore the mechanism of cocarcinogenic or anticarcinogenic solvent action, it seems to us preferable to use in future simple synthetic systems where the conditions are more transparent.

Our present experiments are a first step in this direction. The correlation here observed between rapid elimination of the carcinogen, shortening of the latent period, and increased tumor incidence on the one hand, and between delayed elimination and lengthening of the latent period on the other, is not invalidated by the lack of such a correlation in other more complicated solvents where disturbing factors of a chemical, physical, or biological nature may intervene.

We suggest that one of the main factors determining carcinogenesis is the rate of metabolism of the carcinogen. From the investigations of Chalmers and Peacock (4) and of Berenblum and Schoental (2) the metabolism of 3,4-benzpyrene seems to be predominantly an oxidative process, of which the first stable product is the 8-substituted phenol. At the present time the view prevails that this reaction is a detoxication, and Fieser (7) even postulates a competition for benzpyrene between this supposedly "detoxicating" mechanism and some other unknown reaction that is assumed to be that involved in the actual carcinogenesis. This view is based mainly upon the assumption that the physiological metabolites are noncarcinogenic. This, however, is a question upon which the present evidence is insufficient. There may be stages of oxidation preceding the formation of a phenol, as suggested by Weigert's recent work (21). As to the phenols themselves, those hitherto tested have been for the most part not those formed *in vivo*. Also, the unstable character and greater water-solubility of these compounds make it difficult to maintain a sufficient concentration in contact with the tissues for a sufficient length of time, whereas this is assured if the phenol arises from the hydrocarbon by a steady process of metabolism. From this point of view it is of particular interest that 8-methoxy-3,4-benzpyrene gave no less than 80 per cent of tumors, when injected into mice in a dose of 2 mgm. (3). This is an activity approaching that of the parent hydrocarbon.

If it is assumed, then, that oxidation is an essential condition for active carcinogenesis, our results become understandable. Thus we interpret the cocarcinogenic action of cholesterol as the result of an increased rate of oxidative metabolism of benzpyrene. On the other hand, the presence of phospholipins in the solvent diminishes considerably the rate of oxidative metabolism of benzpyrene, and thus diminishes its carcinogenic activity. Phospholipins are well-known antioxidants, and we have shown in unpublished *in vitro* experiments that the induced oxidation of benzpyrene is in fact strongly inhibited by cephalin. By the study of other antioxidants, acting alone or synergistically (10), it is hoped to obtain further evidence in support of our hypothesis.

Whereas the antioxidant properties of phospholipins suggest a rational explanation for their anticarcinogenic activity, if only in the form of a provisional working hypothesis, the mechanism of the cocarcinogenic effect of cholesterol is quite obscure. Some sterols, such as desoxycholic acid (8) and cholestenone sulphonic acid (23), are known to form molecular compounds with hydrocarbons, thereby increasing their solubility in water; perhaps a similar process is at the basis of the cholesterol effect.

If the interpretation of our results as outlined above proves to be correct, some conclusions as to the actual carcinogen involved are justified. In the phospholipin experiment a considerable quantity of benzpyrene must have lain in contact with the tissues for a long time before any tumor resulted; we assume that the phospholipin inhibited the oxidation of benzpyrene, which is a necessary condition for carcinogenesis. It would therefore appear probable that the hydrocarbon is not itself the true carcinogen, but the metabolite derived from it, presumably as a first oxidation product.

SUMMARY

Three groups of mice were injected subcutaneously with a single dose of 0.3 mgm. of 3,4-benzpyrene dissolved in: (a) tricaprylin, (b) tricaprylin containing 1.5 per cent lecithin and 1.5 per cent cephalin, (c) a 3 per cent solution of cholesterol in tricaprylin. Observations were made of tumor incidence and rate of elimination of benzpyrene. Tricaprylin was taken as the standard solvent for comparison with the others.

The tumor incidence at 20 and 30 weeks was: cholesterol series, 79 and 82 per cent; tricaprylin, 38 and 46 per cent; phospholipins, 12 and 47 per cent respectively. The increased incidence with cholesterol is highly significant ($P<0.01$), but the retardation with phosphatides is not statistically proved ($P=0.1$) and a larger number of observations would be required to establish it. From a consideration of all the evidence it appears probable, nevertheless, that the inhibitory action of phospholipins on carcinogenesis is genuine. The anticarcinogenic effect observed by several authors for various samples of animal fats might well be due to their content of phospholipins. The complex com-

liminary attempts with crude mixtures were not encouraging, and we therefore set out to identify the actual components concerned. Later it may be profitable to study the histological response to simple systems of proved activity.

position of most natural oils makes it preferable to use pure synthetic vehicles where possible, as otherwise the interpretation of results is hardly possible (*e. g.*, with cod liver oil). It is shown that, compared with tricaprylin, the solvents (arachis and sesame oils) previously used by ourselves and others for comparison with mouse fat as solvent, are themselves cocarcinogenic. The differences in carcinogenic activity previously attributed to the anticarcinogenic activity of mouse fat are in part due to this fact.

The rate of elimination of benzpyrene is accelerated when the solvent contains cholesterol ($k=0.027$), and inhibited in the phospholipin solution ($k=0.0062$), compared with tricaprylin ($k=0.016$); the differences in these rates are highly significant ($P<0.01$). The fact that the more rapid elimination of benzpyrene is associated with higher carcinogenic activity, and slower elimination with lower activity, leads us to suggest that, contrary to frequently expressed opinion, the rapidity of elimination of the carcinogen is associated within certain limits with high carcinogenic activity. We suggest that the oxidative metabolism of the carcinogenic hydrocarbon may be a necessary condition for its carcinogenic activity. If this is so, it is probable that an oxidative metabolite of the hydrocarbon is the true carcinogen rather than the hydrocarbon itself.

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Influence of Age on Total Epidermal Lipid During Carcinogenesis Induced by Methylcholanthrene in Mice*

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In our first paper (1) we reported that epidermal cancers induced by cutaneous applications of methylcholanthrene appeared more quickly and in a higher percentage of animals in young than in old New Buffalo mice. The obvious conclusion reached was that the epidermis of these young New Buffalo mice was less resistant to the carcinogenic action of methylcholanthrene than the epidermis of old ones—that an age factor operates.

But in similar experiments young CBA mice developed cancer at almost identically the same rate and in the same percentage of animals as did the older ones. This very conspicuous similarity in the response of these young and old animals indicated that the age factor, if present, was somehow submerged and did not operate. Since the outstanding difference between the New Buffalo and CBA mice is the hereditary difference between the two strains, the conclusion seemed logical that heredity is an important factor in the production of cancer under these particular experimental conditions.

Further comparison of the two series showed that the young New Buffalo mice developed cancer more quickly and in a higher percentage of individuals than did the young CBA mice; whereas the old mice of both strains responded by cancer production in much the same way. Consequently the strain or hereditary factor was strongest in the young, since the young mice of the two strains showed a signal difference in their response to tumor formation by methylcholanthrene whereas the response of the old mice of both strains was essentially the same.

The next step in the study of these age and hereditary factors evidently was to investigate the chemical composition of the reacting epidermises in the hope of finding out whether chemical composition of epidermis conditions the production of cancer. We started to work with calcium because in the major research project of Barnard Hospital (2), which is to integrate the changes that take place in epidermal carcinogenesis induced by methylcholanthrene, a decrease to approximately 50 per cent of normal was observed in epidermal calcium (3); and also for the reason that calcium is a kind of strategic element in physiological processes likely to be associated with basic phenomena such as those of aging.

In this second contribution (4) we first determined the average calcium content in mgm. per 100 mgm. of epidermis, which was as follows:

	New Buffalo	CBA
Young	0.043	0.040
Old	0.054	0.055

Apparently the degree of malignant epidermal response to methylcholanthrene does not depend on the calcium content of the reacting epidermis; for although young New Buffalo mice, with less epidermal calcium, responded more actively than old ones, with more epidermal calcium, the response was about equal in young and old CBA mice despite the difference in epidermal calcium. Again, in these analyses the hereditary factor appeared because the depression of calcium content in hyperplastic stages of epidermal carcinogenesis was greater in New Buffalo mice than in CBA. The decrease in epidermal calcium was about the same in young and old New Buffalo mice despite the differences in response by cancer formation. Moreover, the decrease was less in young than in old CBA mice although the epidermal response was practically the same, so that the concentration of calcium in epidermis does not condition either the speed or the percentage incidence of cancer development.

The experiments herein described relate to the lipid content of the reacting epidermis, and stem likewise from previous work on the major project, in which Wicks and Suntzeff (5) showed that the total lipid-protein nitrogen ratio of the reacting hyperplastic epidermis of young New Buffalo mice decreased 50 to 60 per cent. Moreover, we could think of many

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possible reasons why differences in lipid concentration of epidermis might prove a potent factor. The purpose of these experiments, therefore, was to discover whether any difference exists in the decrease in epidermal lipid in young and old mice of the two strains subjected to the same carcinogen.

EXPERIMENTAL PROCEDURE

Because Wicks and Suntzeff (5) employed the same New Buffalo strain of mice, 3 to 4 months old and treated in the same way with methylcholanthrene in benzene as in our own previous experiments, it did not seem necessary to repeat their work although they determined the total lipid-protein nitrogen ratio whereas we have measured the mgm. of total lipid per 100 mgm. of fat-free epidermis.

Their results are summarized on the left in Table II. It is a simple matter to calculate the percentage decrease in their ratio, as it is the percentage decrease in mgm. of total lipid per mgm. of protein nitrogen.

The method of painting with the carcinogen and the removal of the epidermis from the dermis was the same as that previously employed (1). The total lipid was extracted from the epidermis by refluxing it twice for 2 hours with 25 cc. of a solution containing 3 parts of 95 per cent alcohol and 1 part of reagent grade chloroform. The alcohol-chloroform mixture containing the lipid was then evaporated to dryness on a steam bath, the total lipid re-extracted with petroleum ether (b. p. 30 to 60° C.), and the latter evaporated to dryness on a steam bath in a light weight, 50 cc., glass stoppered, Pyrex Erlenmeyer flask. The total lipid was then weighed to the nearest milligram. As a basis of reference the fat free epidermis was dried at 105° C. to constant weight.

The effect of the benzene alone on epidermal lipid was not investigated, because Wicks and Suntzeff (5) found an average reduction in lipid-protein nitrogen ratio only from 5.25 to 5.17, which is not significant.

RESULTS

The results for old and young mice of the CBA strain are shown in Table I. The mgm. of total lipid per 100 mgm. of dry, fat-free epidermis for the young mice was 29.3, which dropped to 15.0, a decrease of 49 per cent, when the mice had received 3 applications of the carcinogen. The diminution of total lipid of 47 per cent of normal at 17 days after 6 treatments was about the same as that of the group that had received 3 treatments. These results are essentially in agreement with those of Wicks and Suntzeff (3), who found a 50 to 60 per cent decrease in the total lipid-protein nitrogen ratio for hyperplastic epidermis of young New Buffalo mice. In the old age group the amount of total lipid was 32.2 mgm. per 100 mgm. of dry, fat-free epidermis, which fell to 14.7, a decrease of 54 per cent of normal, after the epidermis had been treated 3 times with the carcinogen. However, the total lipid decreased to 10.7 mgm. per 100 mgm. at 17 days after 6 applications, a drop of 68 per cent of the normal. Here there is a greater drop in the total lipid in the old CBA mice than in the young.

The data for the New Buffalo mice are given in

Table I: Total Lipid-Fat Free Dry Weight Ratio of Mouse Epidermis, Strain CBA

Young mice (3 to 4 mos.)						Old mice (12 to 13 mos.)					
No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Mgm. of total lipid per 100 mgm. of fat-free epidermis, mgm.	No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Total lipid per 100 mgm. of fat-free epidermis, mgm.
Normal, untreated mice						Normal, untreated mice					
12			35	109	32.0	9			43	133	32.2
12			36	141	25.5	13			57	194	29.4
12			46	125	36.8	18			102	291	35.0
10			32	118	27.1	40	(total)			Average	32.2
11			36	143	25.2						
57	(total)			Average	29.3						
Methylcholanthrene-treated mice						Methylcholanthrene-treated mice					
10	3	10	26	186	14.0	13	3	10	59	397	15.0
10	3	10	25	185	13.3	10	3	10	43	300	14.3
12	3	10	61	365	19.5					Average	14.7
9	3	10	31	237	13.0						
41	(total)			Average	15.0						
11	6	17	25	164	15.2	8	6	17	22	224	9.8
11	6	17	28	173	16.2	8	6	17	26	219	11.8
22	(total)			Average	15.6	10	6	17	26	249	10.4
						26	(total)			Average	10.7

Table II. The epidermis of old New Buffalo mice contained 32.2 mgm. of total lipid per 100 mgm. of dry, fat-free epidermis, the same as that of the old CBA. Three applications of methylcholanthrene reduced this value to 10.8, a drop of 66 per cent of normal, and 6 treatments to 9.6, a decrease of 70 per cent. The decrease in the total lipid of the old mice of the CBA and New Buffalo strains was almost the same at 17 days, that is, about 70 per cent of normal. On the other hand, the diminution of total lipid in young CBA mice was about 48 per cent of normal. Although our results are not strictly comparable to those of Wicks and Suntzeff, since the latter used protein nitrogen as a basis of reference, the decrease (50 to 60 per cent) that they found for young New Buffalo mice agrees well with our values for young CBA. The effect of benzene alone was not studied, since it has already been shown that the solvent for the hydrocarbon has no appreciable effect on the total lipid (3).

In our second paper (4) we reported that depression in epidermal calcium in both young and old New Buffalo mice was greater than that of both young and old CBA mice, indicating a definite difference in the response as far as calcium is concerned, but this was not linked with a difference in response as measured by tumor production.

The data presented in this, the third contribution, show that the depression in epidermal lipid in both young and old New Buffalo mice was about the same as that in both young and old CBA mice respectively. These observations reveal that the decrease in the total lipid was not paralleled by a difference in epidermal responsiveness to tumor production by the carcinogen.

SUMMARY

The role of total lipid as a factor in the age differences to the response of epidermis in old and young mice of the New Buffalo and CBA strains to methylcholanthrene has been investigated.

The decrease in the total lipid on the basis of dry, fat-free epidermis in the hyperplastic epidermis of old CBA mice was about 70 per cent of normal, while the drop in the young was nearly 50 per cent. In the New Buffalo groups under identical treatment the diminution in total lipid of the hyperplastic epidermis of the old group was 70 per cent of normal, while in the young group the decrease in the total lipid-protein nitrogen ratio was about 60 per cent of normal. No relationship was found between the decrease in total lipid content and the susceptibility to tumor production.

TABLE II

LIPID-PROTEIN NITROGEN RATIO OF MOUSE EPIDERMIS *

YOUNG MICE (3 TO 4 MOS.)

No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Protein N	Lipid-Protein N ratio
		NORMAL, UNTREATED MICE			
8			26.8	5.3	5.06
9			55.8	11.2	4.98
14			61.4	12.0	5.10
12			49.3	10.4	4.74
6			34.9	5.2	6.71
8			50.7	10.3	4.92
57	(total)			Average	5.25
		METHYLCHOLANTHRENE-TREATED MICE			
8	1	10	43.8	20.6	2.13
8	1	10	49.0	15.1	3.24
10	1	10	43.7	19.8	2.21
26	(total)			Average	2.53
5	2	10	40.8	16.2	2.52
8	2	10	44.4	31.1	1.43
7	3	10	49.5	37.1	1.33
9	3	10	45.6	17.6	2.59
5	4	10	43.4	21.5	2.02
11	6	14	54.0	22.9	2.36
4	7	15	40.0	16.2	2.47
49	(total)			Average	2.10

* Data from Wicks and Suntzeff (3).

TOTAL LIPID-FAT FREE DRY WEIGHT RATIO OF MOUSE EPIDERMIS

OLD MICE (12 TO 13 MOS.)

No. of mice	No. of paintings	Time after first treatment to killing of mice, days	Total lipid, mgm.	Fat-free, dry epidermis, mgm.	Total lipid per 100 mgm. of fat-free epidermis, mgm.
		NORMAL, UNTREATED MICE			
11			46	158	29.0
11			49	163	30.0
15			52	185	33.5
15			57	158	36.1
52	(total)			Average	32.2
		METHYLCHOLANTHRENE-TREATED MICE			
9	3	10	35	423	8.2
8	3	10	58	491	11.8
12	3	10	83	721	11.5
29	(total)			Average	10.8
5	6	17	21	268	8.0
9	6	17	23	206	11.1
14	(total)			Average	9.6

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Action of Methylcholanthrene on Certain Scars of the Skin in Mice

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Repair of the necrosis produced in the skin of newborn or adult mice by large doses of ultraviolet rays reveals some histological peculiarities that led us to investigate the action of methylcholanthrene on the scars thus obtained. The same experiment was done later, for comparison, on surgical scars. We report here the results of these investigations.

Newborn Mice

In previous experiments dealing with the influence of oxygen on the radiosensitivity of tissues we irradiated newborn mice, which easily tolerate a general anoxia of the tissues for as long as 15 or even 20 minutes. It was noticed then that the skin of these animals was thin enough to let ultraviolet rays penetrate as far as the deepest layers, where they produced a necrotic radiodermatitis (8, 9). Further irradiation, localized on well defined areas, was then made in order to follow histologically the destruction and repair of the skin.

Technic of irradiation.—The source was a mercury vapor lamp, Biosol Philips, of the mean-pressure type, with active electrodes, stabilized for 103.5 volts and 7.75 amperes. The distribution of energy in the emission spectrum of this lamp had been determined by photoelectric spectrophotometry, by comparison with a standard lamp of the same type, formerly calibrated with great precision (15). The monochromatic flux, $\phi\lambda$, of each emission line, and also of the continuous background (which represents about 10 per cent of the total output) was calibrated in absolute units (ergs cm^{-2} sec^{-1}).

The effects of ultraviolet rays on the skin are highly selective, depending chiefly upon the transmission factors of the keratin layer and the absorption factors of cellular proteins (6). It is important to know for each wave length not only the energetic flux it carries, but also its "efficiency," $E\lambda$. We chose the efficiency factors given by the erythemic curve of Coblentz and Stair (2). The "intensity" of the total radiation output could in that way be measured and expressed in finsen units.[1] We have always used an intensity of 300 finsens per minute.

The mouse, protected against overheating, was shielded by a mantle in which an aperture fixed the limits of the irradiated area (about 30 sq. mm. on the back). In order to choose the dose, we had formerly measured that which produced various phenomena in several strains of mice. The mean values were as follows:

Erythema threshold	200 f.
Definite erythema	600 f.
Epidermatitis	1,000 f.
Necrotic dermatitis	1,500 f.

Photodermatitis produced by one dose of 1,500 finsens, and the process of repair.—Since details of this experiment have already been published (10), we shall summarize here only the facts necessary to an understanding of the subject.

The necrosis, which develops during the first 2 days, affects the entire epidermis and dermis and stops near the subdermal muscle (panniculus carnosus), which may or may not be damaged. It extends to a depth of about 150 μ. Thus it destroys all the hair follicles and sebaceous glands. The adjacent nonirradiated epidermis shows an evident thickening: from the third day on its cells are enlarged and show increased frequency of mitosis. The same occurs in the hair follicles surrounding the destroyed zone: their cells show some hyperplasia, and divide actively.

From the thickened epidermis a thin layer of cells pushes under the necrotic scab and spreads over the irradiated field, which it covers entirely by the seventh day. While it progresses towards the center of this area and thickens, however, the new epidermis is resolved at the periphery into many isolated masses,

[1] For a general treatment of ultraviolet dosage in biological research, see Latarjet (16). The finsen unit is there defined as the dose given on a surface when each square centimeter of this surface receives an energy equivalent to 6,000 ergs carried by 2,967 A. The equivalence is given by the factors $E\lambda$. The "activity," or "intensity" of the radiation is expressed in finsens per minute.

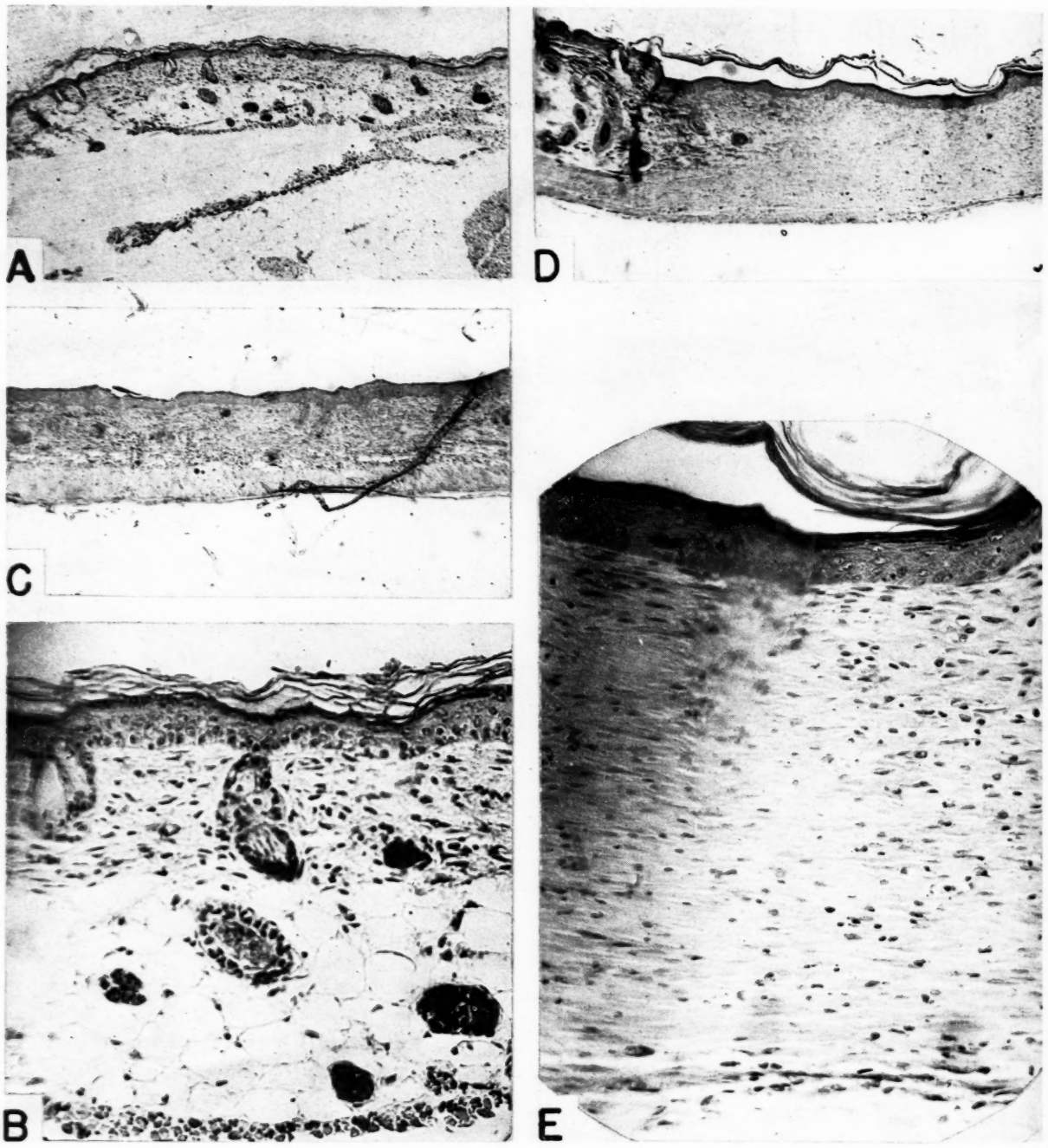

FIG. 1.—A. Newborn mouse 12 days after irradiation. Part of irradiated field where fat has allowed a few epidermal islands to differentiate into newly formed hair follicles. Mag. × 70.

B. Detail of A. In center, new follicle: at left, new sebaceous gland. Mag. × 350.

C. Adult mouse 12 days after irradiation. Some few hair follicles remain in dense dermis. Mag. × 70.

D. Adult after scarring of surgical wound. Skin completely hairless. At left, border with its hypertrophic follicles. Mag. × 60.

E. Detail of D. Mag. × 300.

which make their way into the new dermis, the latter appearing as a narrow strip of dense connective tissue. The destiny of these epidermal islands is uncertain: most of them come against the sclerotic and poorly vascularized tissue of the dermis, and degenerate; a few approach the cutaneous muscle, and sometimes find in the fat that occasionally remains there conditions suitable for their differentiation into newly formed hair follicles (12 days). (Fig. 1, A and B.)

At about the 20th day the scar takes on its definitive aspect: it appears as a thinner zone, with normal epidermis and sclerotic and atrophied dermis; a few scant hairs emerge, irregularly distributed; the scar is enclosed by a border in which the hair follicles and sebaceous glands persist in a hypertrophic condition.

Effects of painting with methylcholanthrene.—Two series of experiments were performed, in one of which (Experiment 1) the applications of methylcholanthrene were started after epidermal scarring was complete; in the other (Experiment 2), before it had begun. We used an 0.3 per cent solution of methylcholanthrene (Hoffman-Laroche) in acetone. One drop was placed at the center of the irradiated area.

Experiment 1.—In 10 mice irradiated 1 day after birth paintings were begun 10 days later, and repeated 3 times a week. The hydrocarbon spread and acted beyond the irradiated area. After about 3 weeks the hairless field extended beyond the scar, but the latter was easily distinguished because of its smooth and pale surface, which contrasted with the rough and reddened aspect of the surrounding zone. Eighty days after paintings had been started the macroscopic lesions began to appear. These nearly always consisted of one or several ulcerations, sometimes covered with scabs, lying on the scar or on its marginal border (Fig. 2). At the same time, or a little later, papillomas appeared occasionally in the nonirradiated zone. The malignant transformation of the ulcers, which occurred in 90 to 120 days, led to large and deep lesions with the external characteristics of rodent ulcer, which evolved very quickly.

Histologically we observed, besides the usual form of epidermoid epitheliomas with many pearls, some nonkeratinizing epitheliomas containing extensive areas resembling perithelium and with a tendency to dissemination of cells.

Experiment 2.—In another series daily paintings were begun the first day after irradiation. Neither the time nor the mode of repair was substantially different.[2] A series of histological examinations showed, however, that the epithelial islands produced during repair of the epidermis regressed entirely. Thus the irradiated field remained completely hairless after scarring (Fig. 1, D and E).

Eight newborn mice were given daily paintings for the first 15 days, and afterwards 3 times a week. The papillomas appeared between the 50th and 217th day. They were located more frequently on the border of the scar; at other times they lay beyond this border, on nonirradiated territory. In no case did a tumor appear on the scar itself, in spite of the fact that the methylcholanthrene had been applied directly to it (Fig. 3). Except in 2 animals, sacrificed on the 54th and the 81st day for histological study, carcinogenesis was observed between the 160th and the 290th days. All these tumors were epitheliomas of the usual cancroid variety.

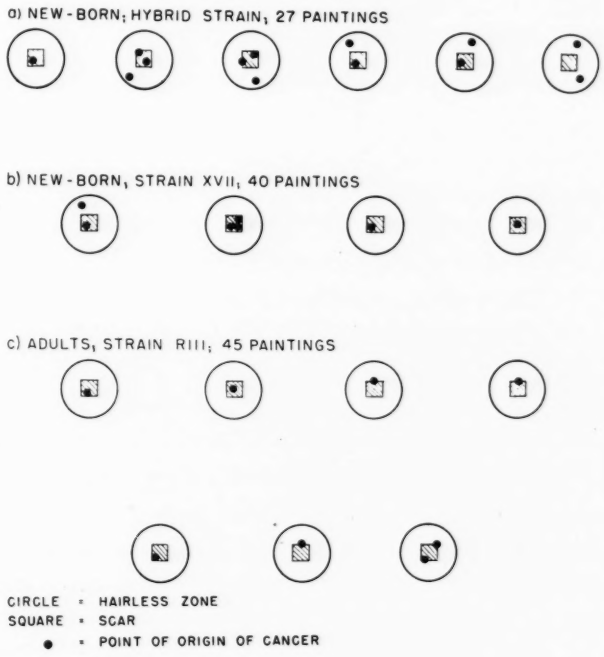

FIG. 2.—Points of origin of cancers in scars containing hair follicles.

To summarize, the results obtained in these 2 types of experiment differ in several respects, not only from each other but also from the results usually obtained with methylcholanthrene. In the first series the earliest lesions appeared on the scar. They were of an ulcerative rather than a papillomatous type, and developed early into epitheliomas poor in keratin, with a strikingly malignant character and an infiltrative growth. In the second series the papillomas appeared most often on the border of the irradiated field, and sometimes beyond this border. They developed into the usual epitheliomatous types. In contrast, the skin of the scar did not undergo the malignant change, as has already been stated.

The only difference revealed by histological exami-

[2] From Haddow's observations the contrary had been expected (5).

nation was, in the first experiment, the presence of newly formed hair follicles on the irradiated field. It seems that these may be regarded as the starting point of the cancers that arose in this scarred area, where they developed into the ulcero-necrotic form well known in the recurrences that sometimes follow radiotherapy. Further, attention is directed to the high frequency of tumors in the border of the scar, where hyperplasia of the hair follicles and sebaceous glands was so common.

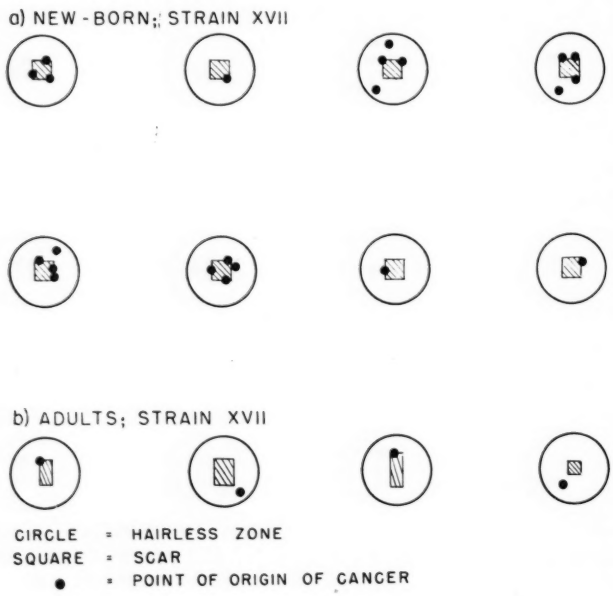

FIG. 3.—Points of origin of cancers in hairless scars.

ADULT MICE

We repeated these experiments with adult mice (12). These, however, oppose to the ultraviolet ray a skin that is about 450 mμ thick and covered with an opaque screen of hair. As any chemical depilation might have introduced a new factor, we resorted to epilation; this was done with a forceps over a dorsal area of about 1 sq. cm. We irradiated 8 days later; at this time the traumatic reaction had disappeared and the new hairs had not yet emerged, so that no shadow was present. The same dose of 1,500 finsens was administered.

Evolution of photodermatitis.—In the adult mouse necrosis again extended to a depth of 150 mμ, so that the deep layers of the skin were unaffected. The hair follicles seemed to be cut. The new epidermis was formed not only from surrounding nonirradiated epidermis, but chiefly from the cut ends of the root sheaths. Underneath, the dermis became sclerotic; it progressively enclosed the remaining follicles, and caused many of them to degenerate. At the end, when scarring was complete, some hairs remained dispersed on the surface of the irradiated area (Fig. 1, C).

Effect of painting with methylcholanthrene. Experiment 3.—Nine mice, 2 months old, were treated under the same conditions as the newborn mice of Experiment 1. Beginning with the 11th day after irradiation 1 drop of methylcholanthrene was placed on the scar 3 times a week. Here again lesions began with ulcerations, starting on the scar or in its border between the 82nd and the 160th day (Fig. 2). Carcinogenesis occurred early (122nd to 183rd day) and the evolution of the epitheliomas was rapid and erosive.

Experiment 4.—In order to reproduce the second experiment in adults, and to control the role played by lack of hair follicles in the scar, it was necessary to destroy all these formations. As ultraviolet rays were unable to reach the deepest follicles, we removed with scissors a circular area of skin 1 cm. in diameter in the dorsal region, separating the skin at the level of its cleavage plane on the muscular aponeurosis. Scarring required about 18 days, and was a two-stage process. During the first 5 to 7 days a connective tissue base was formed in the wound; over this groundwork the epidermis and dermis were repaired during the next 12 days, as they were in newborn animals after photodermatitis. The epithelial islands invaded poorly, however, and soon they all degenerated, so that the field remained hairless. Here, too, the surrounding epidermis showed hypertrophic hair follicles and sebaceous glands with giant hairs. The deep scarring produced a strong retraction, which was more pronounced laterally than in a cephalocaudal direction; hence, in the end, the scar became elliptical and covered an area only one-fifth as large as the initial wound. As in the newborn, painting with methylcholanthrene during the process of repair changed neither its course nor its duration.

Six adult mice, treated as above, were painted 3 times a week with methylcholanthrene, beginning with the 21st day after excision. Two died during the experiment. In the other 4 all tumors started in the border or beyond it, the scar remaining unaffected (Fig. 3).[3] It must be pointed out that 1 tumor was a spindle cell sarcoma.

These experiments confirm the results obtained with newborn mice: (a) tumors originated early and evolved rapidly on the irradiated skin when this was not completely hairless; (b) no tumor developed on skin completely free of hair follicles and sebaceous glands.

[3] These experiments are very similar to those of Brunschwig, Tschetter, and Bissel (1), in which the scar after radium treatment or burning seems to be insensitive to the carcinogenic action of 3,4-benzpyrene, the tumors originating around the scar.

DISCUSSION AND CONCLUSIONS

The facts reported above might lead to a discussion of several cancer problems; for example, the influence of one agent on the malignant process induced by another agent, the histological types of cutaneous epitheliomas, the evolution of tumors growing on irradiated fields, and so on. But we shall consider only their bearing on the two following questions: (a) the role of hair follicles and sebaceous glands in the origin of epitheliomas of the skin induced by chemical substances; (b) the carcinogenic potential of the active repair zone in a wound. These two questions have been discussed for a long time by many authors, on the basis of clinical observations or experimental results. We return to them only in order to present the evidence brought out by our own experiments.

Role of the hair follicles and sebaceous glands in the origin of cutaneous epitheliomas induced by chemical substances.—This relationship, already pointed out by clinicians before the experimental era to explain the localization of occupational cancers, was histologically demonstrated in 1907 by Jores (7) for the epithelial proliferation obtained by intracutaneous injections of scarlet red in oil, according to Fischer's technic (3). In 1916-18 Yamagiwa and Itchikawa (9) were able to follow, after tar painting, the development of follicular epitheliomas, which sometimes turned into sebaceous or trichoepithelial cancers. Finally, the detection of benzpyrene in tissues by fluorescent microscopy verified the concentration of the hydrocarbon in the follicles, and especially in the sebaceous glands (4).[4]

Our experiments suggest once more that these structures play the chief role in the genesis of tumors induced by a chemical substance applied to the skin surface. A possible interpretation of this role is that the substance enters the openings of the hair sheaths and is retained in the follicles. It seems more likely, however, that the liposoluble hydrocarbon diffuses and concentrates in the sebaceous material. This local concentration would explain the higher frequency of cancers arising from the cells of the follicles. It is also possible that some sebaceous products play an additional role in facilitating the malignant transformation.[5]

Carcinogenic potential of the regenerating epithelial border.—This problem is no longer new. The origin of malignant tumors in areas of proliferation is generally recognized; many workers have noticed the high frequency of induced tumors around wounds, and several types of local injuries have been used to favor the formation of a tumor. Our experiments make two striking contributions to this subject.

1. Two newborn mice were irradiated on the right side, then, from the day after irradiation, painted with methylcholanthrene on the irradiated area and on the left side for purpose of comparison. The number of days elapsing before the appearance of papillomas and carcinomas respectively was as follows: in the first animal, 66 and 90 on the right (irradiated) side, 87 and 114 on the left side; in the second animal, 85 and 115 on the right, 120 and 150 on the left. Thus the initiation of the tumor and its evolution went more rapidly on the irradiated side.

2. Twenty-six papillomas were observed in the 12 animals, newborn and adult, painted on the hairless scar (Fig. 3). Of these, 20 started at the border of the scar and only 6 beyond it. This seems too great a difference to be attributed to the application of methylcholanthrene in the middle of the scar. Thus tumors not only appeared earlier, but also occurred more frequently on the border, which was the proliferating strip of repair.

SUMMARY

1. In newborn and adult mice methylcholanthrene was applied to cutaneous fields that had formerly undergone either ultraviolet irradiation with 1,500 finsens, or the removal of a cutaneous disk.

2. Painting a cutaneous wound with methylcholanthrene affected neither the mode nor the duration of repair.

3. A skin zone free from hair follicles and sebaceous glands appeared refractory to the carcinogenetic action of methylcholanthrene. On the other hand, a skin zone repaired after photodermatitis under conditions such that some hair follicles with sebaceous cells had been restored or newly formed, gave rise to rapidly evolving epitheliomas if treated with methylcholanthrene.

4. These experiments emphasize the role of the hair follicles and sebaceous glands in the origin of cutaneous epitheliomas induced by chemical substances, and the high carcinogenic potential of the proliferating strip that underlies the process of repair.

[4] Since coming to this country one of us (R. L.) has learned of a similar observation by Simpson and Cramer (*Cancer Research*, **3**:362-369. 1943; **5**:449-463. 1945.)

[5] This has been considered by Lacassagne and Rudali (14) in the genesis of some experimental cancers of the stomach in mice. Deprived of American literature since 1940, we learned only by chance of the experiments of Simpson, Carruthers, and Cramer (17), according to which methylcholanthrene dissolved in lanoline was inactive. This result seems to be contrary to ours; but do sheep sebum and mouse sebum act in the same way? Moreover, the respective effects of several closely related fat solvents on the carcinogenic action of a hydrocarbon are very variable, and it seems that we cannot actually relate them to any particular property of the solvent (17).

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A Filtrable Agent Producing Lymphoid Tumors and Osteopetrosis in Chickens

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Lymphoid tumors of the chicken have been shown to be readily transplantable by the use of viable cells. Pentimalli (29) and Olson (25, 27) have described the isolation of such strains from what was thought (28) were rare cases of neoplasia, but which had all the characteristics of the condition designated by Feldman and Olson (9) as lymphocytoma. Burmester and Prickett (7) reported the development of similar tumor strains from cases of naturally occurring visceral lymphomatosis. Earlier, Furth (11-13) described the properties of a filtrable agent (Furth strain 2) that produced lymphomatosis, and occasionally myelomas and endotheliomas. Contrary to the results obtained with agents of other chicken tumors such as the endothelioma of Begg and Murray (1), all sarcomas of Rous and Murphy (31), and similar neoplasms (10), Furth's strain 2 agent did not produce a tumor at the site of injection. However, viable neoplastic cells implanted in the pectoral muscle produced lymphomatous tumors similar to those described by Pentimalli (29), Olson (25), and Burmester and Prickett (7).

This report describes briefly the manifestations of a filtrable agent associated with a transplantable lymphoid tumor of the chicken (25).

MATERIALS AND METHODS

The lymphoid tumor strain reported in this study, some of whose manifestations and characteristics have been described previously (5, 6, 25, 27, 30), is identified at this laboratory as RPL[1]-12. The immediate sources of tumor tissue used in these experiments were affected birds in the serial passage of this tumor strain. The transfers were made every 7 days, by injecting a suspension of tumor cells into the pectoral muscle of 6 to 8 week old white leghorn chickens.

Lymphoid tumors in the pectoral muscle and liver used in the preparation of inocula for Experiment 1 were obtained from birds of the 50th RPL passage (inoculum for this passage had been stored at −70° C. for 95 days). The cell-free inocula were prepared from tumor material ground in a mortar with the aid of sterile sea sand to a smooth paste, which was then suspended in 3 parts of 0.85 per cent NaCl solution and centrifuged for 20 minutes at 3,000 r.p.m. The upper three-fourths of the supernatant fluid was carefully transferred to another centrifuge tube with the aid of a slow flowing siphon, and spun for 20 minutes at 3,000 r.p.m. The resulting supernatant was transferred to a serum bottle for inoculation.

Tumors in the pectoral muscle from birds of the 66th RPL passage were used in the preparation of inocula for Experiment 2. The tumor material was first suspended in 3 parts 0.85 per cent NaCl solution with the aid of a mincer (26) and then processed in a bacterial grinder[2] for 2 hours. The resulting suspension was centrifuged for 20 minutes at 19,000 r.p.m. and the supernatant carefully siphoned into a serum bottle preparatory to its inoculation into young chicks. The sediment was resuspended in saline and transferred to another serum bottle for inoculation.

In Experiment 3 blood from birds of the 85th passage with 7 day tumors of the pectoral muscle was used as the source of inoculum. The blood was obtained by cardiac puncture and drawn into a syringe containing 0.1 volume of heparin solution with a concentration of 0.4 gm. in 100 ml. of 0.85 per cent NaCl solution. After the blood cells had been separated in a centrifuge the plasma was filtered through a Seitz sterilizing filter pad. The filter was tested with a broth culture of *Serratia marcescens* and the filtrate found to be sterile. Positive control broth cultures produced excellent growth of bacteria.

Birds that served as positive controls were injected with cellular inoculum, prepared by passing the tumor through a mincer (26), suspending the mince in 3 parts 0.85 per cent NaCl solution, and filtering through a layer of cheesecloth. In Experiment 3 whole blood was used as the inoculum for the positive control birds.

Inoculations were made at 2 and 3 days of age except when otherwise indicated. The amounts used were 0.25 ml. when injections were made intramuscularly and 0.5 ml. for injections into the peritoneal cavity. One per cent fuller's earth (by weight) was added to the centrifuged supernatant injected intra-

[1] Regional Poultry Research Laboratory.

[2] Made by the Unican Instrument Company, Cambridge, England.

muscularly in Experiment 2, and to all inocula injected into the left pectoral muscle in the birds of Experiment 1. The right pectoral muscle of the same birds was injected with the inocula before the addition of the diatomaceous earth.

The pedigreed white leghorn chickens used were obtained from laboratory stock bred (32) to supply chicks that were relatively susceptible to lymphomatosis yet did not develop it when maintained under quarantine. However, progeny of this stock, which furnished most of the chicks used in these experiments, have subsequently showed a significant amount of lymphomatosis even though maintained under the same environment as the parental stock. Thus the chicks may not have been entirely free from infection at the time of hatching.

The chicks were raised in wire batteries for the first 3 months and then transferred to a pen with litter on the floor, where they were kept for the remainder of the experimental period of 6 months. The pens in which the birds were kept were under quarantine throughout the experimental period. All apparatus entering the pens was sprayed with a disinfectant, the feed and litter were handled with precautions against contamination, and persons upon entering or leaving the pens changed shoes and outer garments and used a disinfectant on their hands.

RESULTS

The results of Experiments 1, 2, and 3 (Table I) are similar in that inocula containing viable cells, whether prepared from intramuscular or intrahepatic tumors or from blood of birds bearing tumors, induced the death of all birds inoculated in a short time (average of 10.2 days, including birds injected with centrifuged sediment, Experiment 2). These inoculations produced local tumors in the muscle when the intramuscular route was used, and in the abdominal wall and mesentery when injections were made into the peritoneal cavity. In addition, all birds had extensive involvement of many of the viscera, including the liver, kidney, spleen, pancreas, proventriculus, and gonad.

Chicks inoculated with cell-free preparations, whether these were obtained by the use of a centrifuge (13) or by filtration, did not show evidence of tumor formation (even though a cell irritant, fuller's earth, was used) or other disease until they were at least 10 weeks of age. At this time clinical symptoms resembling osteopetrosis began to make their appearance in all 3 experiments, and by 6 months of age an average incidence of 41 per cent was obtained. During the same period, 56 per cent of the birds inoculated with cell-free material developed macroscopic tumors of the viscera. Most of them had massive tumorous involvement of the liver. Out of a total of 80 birds inoculated with cell-free material, 84 per cent developed tumors of the viscera, bone, or nerve in 6 months' time, to the extent that diagnosis could be made by gross examination. Of the 67 birds that were positive, 20 had osteopetrosis without gross evidence of other pathological lesions, 29 had visceral tumors without osteopetrosis, and 16 had a combination of osteopetrosis and visceral tumors. The remaining 2 had neurolymphomatosis. Only 1 of the 29 birds with tumors of the viscera had gross nerve changes typical of neurolymphomatosis.

The average age at death of birds that died with liver or bone involvement in the experimental period of 6 months was 144.2 days for those inoculated with cell-free material, in contrast to only 10.2 days for those given cellular inoculum. A few of the surviving birds, some with and some without osteopetrosis, were inoculated with a suspension of RPL-12 tumor cells and found to be still susceptible to local tumor development and metastasis to the viscera.

The 15 chicks of Experiment 2 that were not inoculated were raised in the same brooder space, and allowed to intermingle with inoculated birds during the entire course of the experiment. One bird at 67 days of age and another at 89 days showed clinical symptoms of neurolymphomatosis. They were killed and the diagnosis was confirmed at necropsy. At the termination of the experiment one bird, which seemed normal clinically, showed lymphomatous tumors of the liver, heart, and spleen at autopsy. Thus a total of 3 cases of lymphomatosis appeared among 15 birds raised in contact with the inoculated birds.

In the third experiment the noninoculated controls were kept in the same pen but in a separate battery for the first 3 months, thus eliminating the chance for direct contact; however, there may have been indirect contact through the air, dust, flies or other vectors. No evidence of any of the manifestations of the avian leukosis complex (17) was found in any of these controls. Only one death occurred, and that was due to trauma of the poll region caused by cannibalism by the pen mates.

Pathological Manifestations

Osteopetrosis.—This condition was easily recognized by clinical examination because the shanks were almost always affected. In the early stages the metatarsus showed irregular surfaces, a convexity of the anterior outline, or a thickening of the diaphysis. Irregular enlargement of other long bones of the leg or wing were detected by digital palpation. The increased diameter of the metatarsal bones resulted in immobilizing the dermal structures, which made them harder to the touch, and they seemed to have an increased surface temperature.

The metatarsus and tibia were found to be the most frequently enlarged; however, almost all the other bones showed similar derangement. The change was usually, but not always, bilateral, and the extent and number of bones that showed gross enlargement seemed to depend upon the duration of the disease.

The bones had a rough, irregular, porous surface, which was covered by a hypertrophied periosteum (Fig. 1). The porous appearance of the bones was due in part to irregularity of the periosteum and to peripheral marrow spaces. On gross examination the osteopetrotic changes seemed to be largely confined to the diaphysis. The marrow cavity was reduced in diameter by abnormal deposition of spongy bone. The bones of some birds showed no enlargement but had a slight surface irregularity and yellow opaque areas, which were believed to be less advanced stages of the disease.

Histological examination of a few cases with the characteristic osteopathy indicated that important changes occurred in the periosteum. Hyperplasia of the periosteal tissues resulted in thickening of this layer with apparent formation of new and abnormal cancellous bone (Figs. 5 and 6). Numerous irregularly placed cavities containing hyperplastic tissue, which appeared to be bone marrow, were found throughout the cancellous structure. The normal diaphyseal architecture was apparently completely altered.

Visceral tumors.—The disease in birds that died with visceral tumors ranged in type from the acute to a relatively chronic form. The acute type was characterized as follows: (a) the absence of clinical symptoms except during a few hours prior to death, when they might appear weakened and lethargic; (b) the birds were usually in good flesh; and (c) the organs affected were usually diffusely involved by tumor, the liver being friable (Fig. 4).

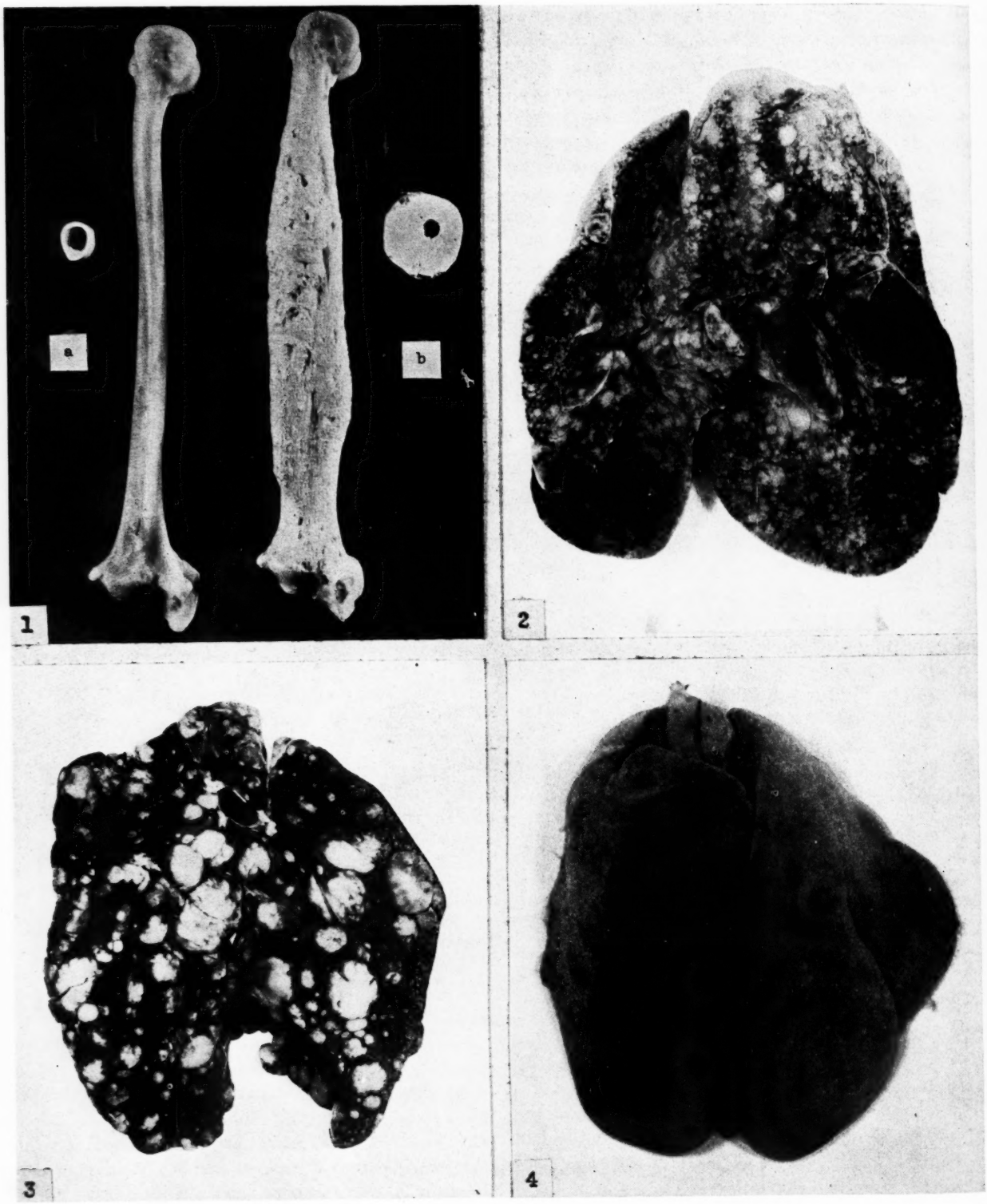
Birds with the chronic type of the disease usually showed symptoms of weakness for several weeks prior to death. They became emaciated, but usually had a

TABLE I: RESULTS WITH CELL-FREE AND CELL-CONTAINING INOCULA PREPARED FROM VARIOUS ORGANS OF BIRDS WITH LYMPHOID TUMORS OF STRAIN RPL-12

Inoculum	Route of inoc.	No. birds	Number with tumors					Average survival, days
			Site of inoc.	Bone †	Viscera †	Nerve	Total	
EXPERIMENT 1								
Muscle tumor, cell suspension	im.*	7	7	0	7	0	7	7
centri. supernat. of ground tumor	im.*	5	0	4	2	0	5	122
	ip.	6	0	4	3	0	6	119
Tumorous liver, cell suspension	im.*	7	7	0	7	0	7	10
centri. supernat. of ground tumor	im.*	7	0	5	3	0	5	156
	ip.	5	0	1	4	0	5	138
EXPERIMENT 2								
Muscle tumor, cell suspension	im.	4	4	0	4	0	4	8
centri. sediment	im.	15	15	0	15	0	15	13
" supernat. of ground tumor	im.*	15	0	4	9	1	12	150
	ip.	14	0	6	6	1	10	157
Not inoculated (contact)		15	0	0	1	2	3	‡
EXPERIMENT 3								
Whole blood	iv.	4	0	0	4	0	4	7
" "	ip.	4	4	0	4	0	4	13
Plasma, Seitz—filtered	iv.	14	0	6	8	1	12	132
	ip.	14	0	6	10	0	12	165
Not inoculated (contact after 90 days)		14	0	0	0	0	0	

im. = intramuscularly
ip. = intraperitoneally
iv. = intravenously

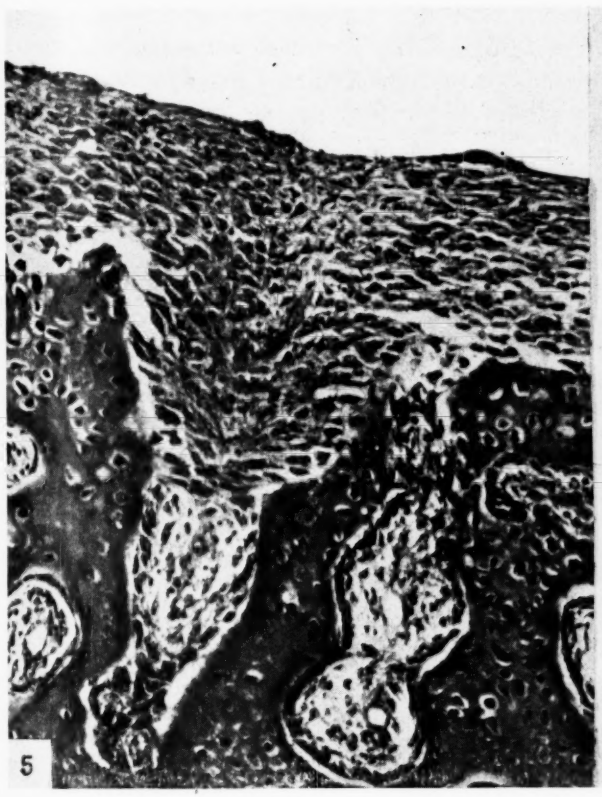
* Fuller's earth added to inoculum.
† Cases with tumors of the bone and viscera appear in both columns.
‡ All were killed.



FIGS. 1-4

full or protruding abdomen and often grew pale. Affected viscera usually had nodular or focal tumefaction. The livers of such birds often had comparatively large tumor nodules (1 to 2 cm. in diameter) separated by apparently normal parenchyma or by small focal tumors (Fig. 3). Some livers were a mass of innumerable small focal areas, leaving very little visible liver parenchyma (Fig. 2). Such a tumorous liver was firm, friable, and somewhat resistant to sectioning. The liver in these cases was often as much as four times the normal weight. In birds with the acute type of illness the liver was enlarged, but usually not more than twice the normal size. Many gradations and variations between the two types were found. Almost all the other viscera were occasionally found to be affected. The frequency of gross involvement of the viscera in the 45 cases with visceral tumors identifiable at necropsy is given in Table II.

The histological changes observed (Fig. 7) in the viscera were similar to those described by Olson (25), Burmester and Prickett (7), Jungherr (18), and to certain cases of naturally occurring lymphomatosis (30). However, they differed in showing more local leukemoid reactions in the viscera, and by the occurrence of focal areas of necrosis in the liver, probably associated with infarctive processes related to the tumefaction.



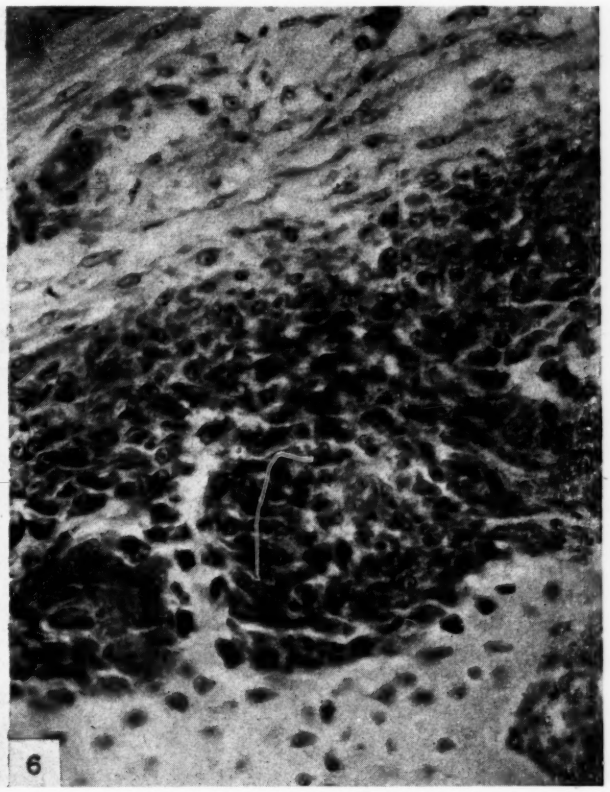


FIG. 5.—Cross section of osteopetrotic tibial shaft showing hyperplasia of periosteum and its extension into deeper bony structure. Numerous spaces containing hyperplastic marrow seen throughout. Hematoxylin-triosin. Mag. × 243.

FIG. 6.—Higher power photograph showing pronounced cellular activity within periosteum. Hematoxylin-triosin. Mag. × 505.

DISCUSSION

Young chicks inoculated with a cell suspension of the RPL-12 lymphoid tumor developed local tumors with visceral metastases, and died in an average of

DESCRIPTION OF FIGURES 1 TO 4

(Mag. × 0.65, approx.)

FIG. 1.—(a) Tibia of normal chicken. (b) Tibia of osteopetrotic bird inoculated with tumor filtrate 162 days before death.

FIG. 2.—Diffusely distributed tumor foci in liver of bird inoculated with filtered plasma 145 days before death. Weight, 230 gm.

FIG. 3.—Nodular tumor formation in liver of bird that died 166 days after inoculation with extract of tumor of muscle. Weight, 187 gms.

FIG. 4.—Liver of bird killed at termination of Experiment 3, showing typical diffuse involvement. Weight, 150 gms.

TABLE II: GROSS INVOLVEMENT OF VISCERA IN 45 POSITIVE CASES THAT RECEIVED CELL-FREE INOCULUM

Lymphomatous organ	Per cent of cases
Liver	93
Spleen	82
Kidney	58
Gonad	31
Heart	22
Serosa	13
Pancreas	9
Proventriculus	9
Adrenal	4
Intestine	4

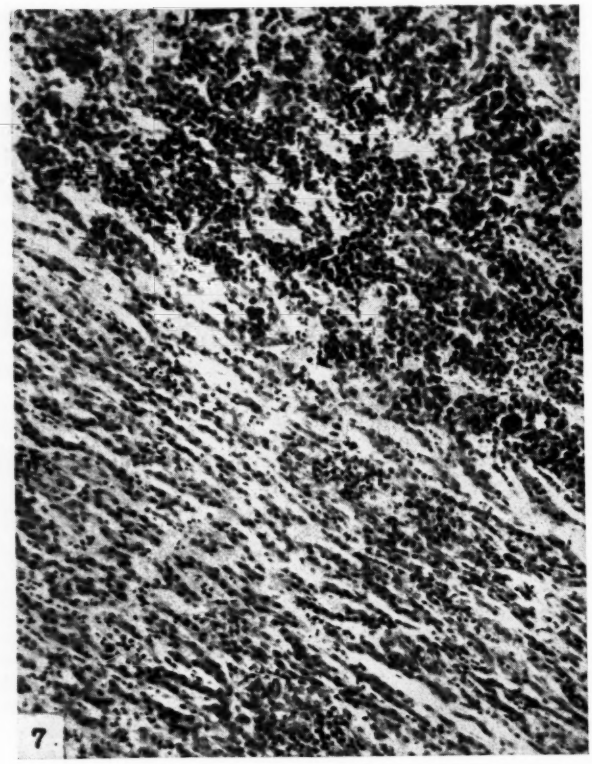


FIG. 7.—Peripheral zone of hepatic lymphomatous nodule showing character of lesion and its influence on adjacent liver parenchyma. Hematoxylin-triosin. Mag. × 165.

10.2 days. Extracts or blood plasma from the same donors made cell-free by either centrifugation or filtration did not induce local tumors or visceral involvement in a short time, but produced visceral tumors and osteopetrosis after an incubation period of 2 to 6 months. The tumefaction of the viscera obtained with the cell-free inocula varied from the diffuse, friable, acute type to the focal, nodular, chronic type. With the differences already noted, the lymphoid tumors were similar in their gross and microscopic appearance to those observed in other malignant tumor strains (7, 25, 29), and to some cases of lymphomatosis (19, 30). The osteopathy described in these experiments was similar in its gross manifestations to the osteopetrosis occurring naturally (2, 16, 20) and following inoculation (4, 8, 16), and the histological changes resembled the osteopetrosis described by Jungherr and Landauer (16). Brandly, Nelson, and Cottral (4) state that augmented cellular activity of the periosteal region, together with the presence of irregular spaces in the bony structure, is characteristic of tarsometatarsal involvement. These changes were typical of the histological alterations found in the experiments described. This is particularly true of the extensive hyperplasia of the periosteal tissues and the lack of medullary fibrosis. Similarity in its transmission characteristics to the osteopetrotic strains previously described (4, 16) was further demonstrated when the blood of an osteopetrotic case of Experiment 1 was injected intravenously, intramuscularly, and intraperitoneally into 45 chicks 10 days of age. Osteopetrosis developed in all 3 groups, giving a total of 24 cases at 96 days of age, when all birds were brought to necropsy. Eleven of the 24 had lymphomatous tumors of the viscera in addition to the osteopetrosis, and 4 others had visceral tumors without gross evidence of osteopetrosis. This is a much higher incidence of osteopetrosis, and at an earlier age, than was obtained by Jungherr and Landauer (16); Brandly, Nelson, and Cottral (4); or Duran-Reynals (8).

That the osteopetrotic propensity is not found in all avian lymphoid tumor strains is indicated by the finding that no osteopetrosis was obtained under similar experimental conditions when strain RPL-16 was used as the source of inoculum. When Seitz-filtered plasma or muscle-tumor extract of an RPL-16 donor was injected into young chicks, 39 per cent developed visceral tumors in the age period of 60 to 180 days, but contrary to the results obtained with RPL-12 no osteopetrosis appeared. Although strain RPL-16 is similar to RPL-12 in many of its manifestations, its source and origin were quite different (7).

Osteopetrosis was not observed by Furth (11-13) in his strain 2, which in other respects was similar to RPL-12 in that inocula containing viable cells induced tumor growth at the site of inoculation whereas cell-free or filtered extracts produced no local reaction but resulted in a systemic disease with lymphomatous tumefaction of the viscera. Furth occasionally observed myelomas and endotheliomas, which were not identified in birds inoculated with strain RPL-12. The latter difference, however, may be due to a lack of unity in nomenclature, or interpretation and identification of the significant cell type. With strain RPL-12 there was a distinct difference in the survival period of birds inoculated with cellular as compared with cell-free inocula. Birds that received cellular inocula died, on the average, in 10.2 days, whereas

those that received cell-free material died in 144.2 days (average). Furth does not indicate that he obtained a comparable difference with his strain 2; however, the data presented (11, 13) indicate that the difference, if any, was much less.

Lesions resembling human osteodystrophia fibrosa cystica were obtained by Oberling and his associates (21-24) in birds maintained in outdoor cages on a mineral-poor diet. Gohs (14, 15) produced similar lesions, but without parathyroid hyperplasia, by repeated injections of normal embryonic or adult avian bone marrow treated with x-rays and glycerin. Brandly (3) injected embryonated eggs with whole blood and washed red cells; in 7 to 10 days the spleen, liver, and the diaphysis of the long bones of the legs and wings became enlarged. The microscopic alterations found in the periosteal region were similar in many respects to those obtained in the inoculations described in this report. Gross lesions were the same, irrespective of whether the blood was from apparently normal birds or from birds having lymphomatosis or erythrogranuloblastosis; however, they were absent when cell-free plasma or blood cells killed by x-rays or distilled water were used. Thus similar hypertrophic osteopathies may result from a wide variety of treatments or circumstances, which emphasizes the need for caution in assigning their etiology.

Olson (25, 27) apparently did not obtain bone lesions in the serial passage of this tumor strain by transplantation, nor did they appear while the tumor was maintained in serial passage at this laboratory. Why osteopetrotic alterations developed with cell-free extract but not with cell suspensions is not apparent. The age of birds at inoculation and the dosage used may have been influencing factors, since all serial passage inoculations were made with birds 4 or more weeks of age; whereas the birds of these experiments were inoculated at 2 or 3 days of age. In an immunity experiment to be reported elsewhere, chicks 2 days of age were inoculated intramuscularly with a suspension of cells. The dosage, however, was only 1/40,000 of that used in serial passage and in cell free transmission inoculations. Second and third inoculations were made with a much larger dose, at 23 and 64 days of age respectively. Tumors developed at the site of the first inoculation but no bone lesions were found, even though many of the birds lived over 300 days. However, a high percentage of them developed lymphomatous tumors of the viscera indistinguishable from those elicited by cell-free extracts.

Three birds of 15 uninoculated controls developed lymphomatosis when they were mixed with the inoculated birds at the time of inoculation (Experiment 2); however, no birds of the same families showed evidence of disease when they were brooded separately for the first 3 months. Thus it may be suggested that the agent was transmitted by direct bird-to-bird contact at some time prior to 3 months of age. However, the suggestion is of less significance when we consider the fact that of the 3 birds giving positive results in the contact control group 2 had neural involvement, while only 2 of 22 giving positive results in the inoculated groups had the neural form. That the 3 positive cases were the result of transmission of the agents through the egg remains a possibility, as indicated previously.

The data presented suggest that whereas the transplanted tumor cell is responsible for tumors that develop in a short time (7 to 10 days) at the site of inoculation, with metastasis to other tissues, a filtrable agent or agents will produce lymphomatous tumors of the viscera and osteopetrosis after a much longer incubation period (2 to 6 months) without inducing a tumor at the site of inoculation.

Whether osteopetrosis and the visceral tumors are due to a single entity or result from the action of more than one agent cannot be ascertained from these data. The majority of cases had gross osteopetrosis or visceral tumors, and a combination of the two occurred in only 24 per cent of the total positives. Microscopic examination of 6 osteopetrotic cases without gross tumefaction of the viscera showed that none had lesions typical of lymphomatosis.

The high incidence of visceral tumors obtained without evidence of osteopetrotic manifestations, in birds inoculated with the filtrate of another lymphoid tumor strain (RPL-16), would suggest that the two manifestations are caused by separate entities or that agents of the two lymphoid tumor strains are different. A relation in the occurrence of osteopetrosis and lymphomatosis (including lymphomatous visceral tumors) was noted by Jungherr and Landauer (16) and by Brandly, Nelson, and Cottral (4). It was suggested (16) "that certain transmissible strains of lymphomatosis may have an overt or latent power of bringing about osteopetrotic alterations in various degrees." Both reports state that a complete identity of etiology seems unlikely.

Since the filtrable agent was obtained from a strain after it had been propagated through 200 serial passages by the transplantation of viable tumor cells, it may be suggested that the agent was also thus propagated within or in close relation to the neoplastic cells. Furthermore, relatively large amounts of it must soon have appeared in the blood stream, since filtered plasma from a bird 8 days after intramuscular implantation produced as many positive cases as did centrifuged extracts of the primary tumor.

SUMMARY

1. Manifestations of the filtrable agent or agents of a transplantable lymphoid tumor of the chicken were demonstrated in 3 experiments involving 150 birds.

2. Inocula containing viable tumor cells induced tumor growth at the site of inoculation, metastasis to the viscera, and death of all birds in a relatively short time (average 10.2 days); whereas centrifuged extracts of the same tumors, or filtered plasma of birds bearing tumors, when injected intramuscularly, intraperitoneally, or intravenously into 2 to 3 day old chicks, induced in 6 months a high incidence of osteopetrosis and lymphomatous tumors of the viscera (average of 81 per cent on gross examination) but no tumors at the site of inoculation.

3. These results suggest that the avian lymphoid tumor strain under study, which has been transferred serially in over 200 passages by transplantation of its cells, carries with it a filtrable agent or agents capable of inducing osteopetrosis and lymphomatous tumors of the viscera after an incubation period of at least 2 months.

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Ocular Tumors with Exophthalmia in Xiphophorin Fishes*

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Many types of spontaneous neoplasms are found in fishes (10). Melanotic tumors, described histologically by Reed and Gordon (12) and by Gordon and Smith (6), are produced genetically in hybrids by selective matings between the Mexican swordtail and the spotted varieties of the platyfish. The specific regions of the body where these tumors are likely to develop, such as the dorsal, ventral, or lateral areas, are determined by a series of definite genetic factors according to a preliminary statement by Gordon (5).

The available literature on tumors in cold-blooded vertebrates fails to reveal a paper on ocular tumors that induce exophthalmia, or "pop-eyes," with perhaps the exception of Plehn's description of a myxosarcoma of the orbit, with considerable displacement of the globe, in the eye of the European tench (*Tinca vulgaris*) (11). Johnstone (7), too, described a flounder (*Pleuronectes flesus*) with protusion of one eye which he ascribed to a lymphosarcoma.

This report will describe tumors of the eye in 5 fishes, 4 of which were reared under similar laboratory conditions and the fifth outside our laboratories. This suggests that the eye tumor disease is not solely the product of our cultural practices, although the latter may be involved as contributary factors. The infrequency of ocular tumors and the ease with which they may be overlooked are responsible for the lack of knowledge on this highly important problem.

The purpose of this paper is to call attention to these ocular tumors in the more primitive vertebrates, to present some basic observations on their structure, and to suggest their probable region of origin. Two of the fishes studied had tumors in both eyes, and exophthalmia was induced in each by the presence of extra masses on the inner surfaces of their eyes, which involved the choroid completely. The other specimens had unilateral ocular tumors associated with edema, hemorrhage, and separation of the retinal layers. All these conditions contributed to the production of exophthalmia in one or both eyes.

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MATERIAL AND METHODS

With one exception the fishes used in these studies were obtained from the Fish Genetics Laboratory of the New York Zoological Society, maintained temporarily at the American Museum of Natural History, and were representatives of the *Xiphophorini,* a Mexican tribe belonging to the *Cyprinodontes,* an order of teleost fishes. They were maintained in 5-gallon aquaria in temperature-controlled rooms adjusted not to fall below 22° C. The tumorous fishes were found in aquaria in widely separated sections of the laboratory, in most instances singly among 10 to 20 of the same brood. Only 4 of 500 aquaria contained fishes with ocular tumors, which indicates a relatively uncommon condition of sporadic occurrence.

The fishes in all our aquaria were fed the same diet, consisting of commercial fish foods that were made up for the most part of dried shrimp, meat by-products, and cereals. These rations were alternated with a paste composed of fresh beef-liver juice to which sufficient Seravim cereal had been added to thicken it. Small portions of thoroughly cleansed Tubifex worms were added to the diet in season.

The fishes that developed ocular tumors were found among 5 groups, and the number of individuals affected was as follows:

1. The common platyfish (*Platypoecilus maculatus*); New York Zoological Society Culture No. 160; 1 out of a brood of 20.

2. The pygmy swordtail (*Xiphophorus pygmaeus*); New York Zoological Society Culture No. 4[4]; 1 out of a brood of 16.

3. A platyfish-swordtail hybrid, "A" (*P. maculatus-X. hellerii*); New York Zoological Society Culture No. 127 × 127-11; 6 out of a brood of 15.

4. A platyfish-swordtail hybrid, "B" (*P. maculatus-X. hellerii*); New York Zoological Society Culture No. 133 × 127-21; 2 out of a brood of 16.

5. A platyfish-swordtail hybrid, "C" (*P. maculatus-X. hellerii*); presented to us from the private aquarium of Mr. Fred Flathman, Woodhaven, Long Island, N. Y.

All the exophthalmic fishes were full grown, at least 6 months old, and some over a year old before the eye condition developed. The normal, or average, life span for these fishes is about 1½ years.

The fishes selected for study were detected not only by the protrusion of one or both eyes, but by their abnormal behavior. They were not isolated but permitted to remain in the aquaria until signs of blindness and loss of power became evident, when they were carefully removed from the tanks without struggle or injury and placed *in toto* in Bouin's fixing fluid. The fixation period rarely lasted more than 18 to 20 hours. The larger fishes were then cut into 3 parts; the head was severed below the gills and the second section made just posterior to the anus. Fixation process was followed by decalcification in 5 per cent nitric acid; a more favorable decalcifying fluid was made of 20 per cent sodium citrate with an equal quantity of formic acid (sp. gr. 1.20, approximately 85%). Sections 5 to 7μ thick were made parallel to the dorsal surface of the head, while the mid-portion was cut at right angles to the axis of the anterior part and parallel to its lateral surface. These sections were made to determine the presence of possible metastases and for cytogenetic studies now in preparation.

Serial sections were made of all heads studied. Some were stained in Fleming's triple stain, some in Delafield's hematoxylin with eosin, and some in van Giesen's stain together with a combination of dyes that gave great contrast when properly employed. This last method consisted of staining in gentian violet solution and counterstaining with orange G dissolved in oil of cloves; the process is observed under the microscope until the cytoplasm and nucleoplasm assume a golden-yellow color and the nuclear granules a delicate violet-gentian. This has been used for other animal tissue with satisfactory results (8).

Symptoms and Behavior

The exophthalmia due to ocular tumors in xiphophorin fishes, which has not been described hitherto, appears to develop gradually, for young fishes show no morphological ocular disturbances and their behavior is normal. Later, when one of the eyes definitely extrudes, blindness on that side is evident from the fish's abnormal movements; it swims in a circle with its good eye toward the center, and has a tendency to remain quiescent for long periods of time, resting on the bottom of the aquarium or on an aquatic plant. In this condition, when its extruded eye is turned toward the observer, the fish will not move if the shadow of one's hand is passed before it, whereas a normal fish would dart away instantly under the same stimulus. If the glass of the aquarium containing the exophthalmic fish is tapped lightly the fish seems to arouse itself from its lethargy and spins around frantically, often bumping into the sides of the tank and dropping heavily upon the bottom. In its terminal stage the fish has a tendency to lose its sense of equilibrium, lies on its side, and is incapable of responding to external stimuli.

The Normal Eye

The normal teleost eye, figured and described by Walls (13), is representative of the *Cyprinodontes,* and the species *Xiphophorus* and *Platypoecilus* and their hybrids used in these experiments agree in most of the representative structures observed in these teleosts.

A longitudinal section of a young normal albino hybrid (*X. hellerii* × *P. maculatus*), 24 hours old and 8 mm. in length, is shown in Fig. 1A, and a radial section of its eye in Fig. 1. Similar views of a normal gray hybrid from the same brood are presented in Figs. 2A and 2. The radial section represents the near temporal portions of the eye. The posterior portion in these fishes consists of a slightly elongated globular body, with its long diameter parallel to the lateral surface of the head. The retinal layers alone assume this configuration and are covered anteriorly by a more or less flattened cornea. In sections the sclera and choroid appear to form loose layers of tissue about the retina, and are not in contact with each other or with the pigment layer of the retina.

The sclera forms a thin cartilaginous cup, with its fundal portion taking on a fibrous nature. The inner layer of the cornea is continuous with the sclera, and covered by the epidermis forming the epidermal cornea. The choroid is a loose cellular body following in form the curvature of the retina; its deepest portion is usually thicker and tapers off to a thin layer as it approaches the iris. In gray and in black hybrids both surfaces of the choroid may be covered with melanin granules, forming a thin coat of heavily stained material.

The iris in the albino fish is not entirely devoid of pigment, but in the gray or black hybrids melanin forms a compact layer on the inner, and thinner layers on the outer surfaces, with scattered connecting bands between the two (Figs. 2A, 2). The retina conforms to the structure described for the generalized teleost fish. The lens is globular, and no mechanism for accommodation has been found.

The Ocular Tumors and Exophthalmia

Platypoecilus maculatus.—Tumors were not suspected as the cause of exophthalmia in this fish until sections of the head were made. In *P. maculatus,* reported below, only slight bilateral exophthalmia was

observed. The "pop-eye" condition was sufficiently evident to attract attention. There was no evidence of visual disturbance in this fish.

Sections (Figs. 3, 4) made through the eyes parallel to the dorsal surface of the head revealed two large masses of tissue lying exterior to the scleras of both eyes and within the space bound by the ocular orbital cartilaginous bones of the head. The scleras appeared normal, but the choroids in both eyes lay in close proximity to the retinal pigment layer. The choroids were much thickened, and contained deeply staining patches of tissue somewhat regularly spaced from each other. The large tumor masses lying outside the scleras also presented these deeply staining patches of tissue. Here, however, these patches were larger and more compact than in the choroid tissue proper.

In the right eye the continuity of choroid and tumor is seen, the two being joined by a small bridge of loose tissue of less densely staining type (Fig. 3).

A section approaching the ventral surface of the head and the right eye (Fig. 4) demonstrated clearly the intact and uninvaded layers of the retina; here the densely stained choroid growth lay adjacent to the retina, while the large tumor was exterior to the sclera. The perforation of the sclera by the neoplasm (Fig. 3) was not extensive, for no trace of its continuity was seen in sections at lower or higher levels. The same condition prevailed in the left eye.

The tumors in both orbital spaces were more or less cup-shaped, filling the spaces between the eye and the protective cartilages. The fundal portions were thick and tapered off to the side in a layer of tissue that extended to, and invaded, the corneal region.

The tissue in the choroid region (Fig. 5) inside the sclera and in close proximity to the pigment layer of the retina appeared as a wide, though loose, reticulum consisting of epithelioid cells, fibroblast, and thread-like bodies, together with large melanophores and dispersed melanin granules; large masses of red blood cells were present. The thick layer of choroid tissue was subtended by a thin, though densely pigmented, layer of melanin. The densely staining, compact tumor masses (Fig. 6) imbedded in the choroid were similar in structure to those found in the tissue beyond the sclera, and were made up primarily of epithelioid cells impregnated with granules of melanin; under higher magnification (Fig. 7) these elements were seen to assume a pearl-like, or fasicular, orientation or configuration.

The cytoplasm of the epithelioid cells formed a thin, delicately stained border about a relatively large nucleus, which was somewhat elliptical or globular in shape and surrounded by a densely stained membrane; the chromatin was sparse and appeared in fine strands, most frequently distributed about the nuclear membrane. Blood cells were frequently found scattered through the tumor. The tumor was bounded by a membranous layer of tissue two to three hexagonal-shaped cells thick. Degeneration of these compact masses of epithelioid cells was apparent, and there was a tendency for the cells to separate from the large masses and form independent, densely staining clusters.

This tumor was obviously choroidal in origin, and seemed to be made up of a loose, fibrous stroma supporting chiefly epithelioid cells. It seemed to be analogous in structure to melanomas of the human eye, as described by Callender and Wilder (1) and by De Coursey and Ash (2).

The malignancy of this tumor is unquestionable, yet no metastatic activity seemed to be in evidence. Serial sections of this and other fishes failed to reveal any overgrowth of a neoplastic nature in the internal organs.

Xiphophorus pygmaeus (Pygmy swordtail).—This fish presented a pedunculated, irregular, globular mass of tissue that completely involved the right eye. The movements of the fish were aberrant, which indicated that it was at the terminal stage of its disease. The fish was fixed alive and sections of the entire body were made parallel with the dorsal surface.

The photograph of such a section through the axis of the right eye appears in Fig. 8. Both eyes are aphakic due to mechanical difficulties, yet the tumor was not unusually disturbed. The visual axis of the right eye was blocked entirely by a globular mass of tumor that completely surrounded the eye, except for a small opening in the optic nerve region. There appeared to be considerable displacement of scleral tissue. The dark band on the outer surface of the neoplasm was of a cartilaginous nature and resembled portions of the sclera. The choroid lay in contact with the retinal pigment layer, which appeared intact. It was thick and fibrous, with strands of melanin running through it (Fig. 9).

Microscopic examination of the growth revealed disoriented masses of spindle-shaped cells (Figs. 9, 10), which formed the main body of the tumor and were peppered with melanin-like granules; lightly in some areas, more densely in others. The cells were arranged in broad fascicles, and their poles took various directions. In the uveal area they were interspersed with clusters of epithelium-like cells, which alone appeared to be undergoing karyokinesis. These cells, like the spindle-shaped cells, were arranged in fascicles (Fig. 11). The epithelioid cells were round, with centrally placed, well-differentiated, nucleoli. The nuclear membranes and the fine chromatin granules took the gentian violet dye in a characteristic manner. The nuclei were circular in outline with narrow borders of cytoplasmic material. The cells were more

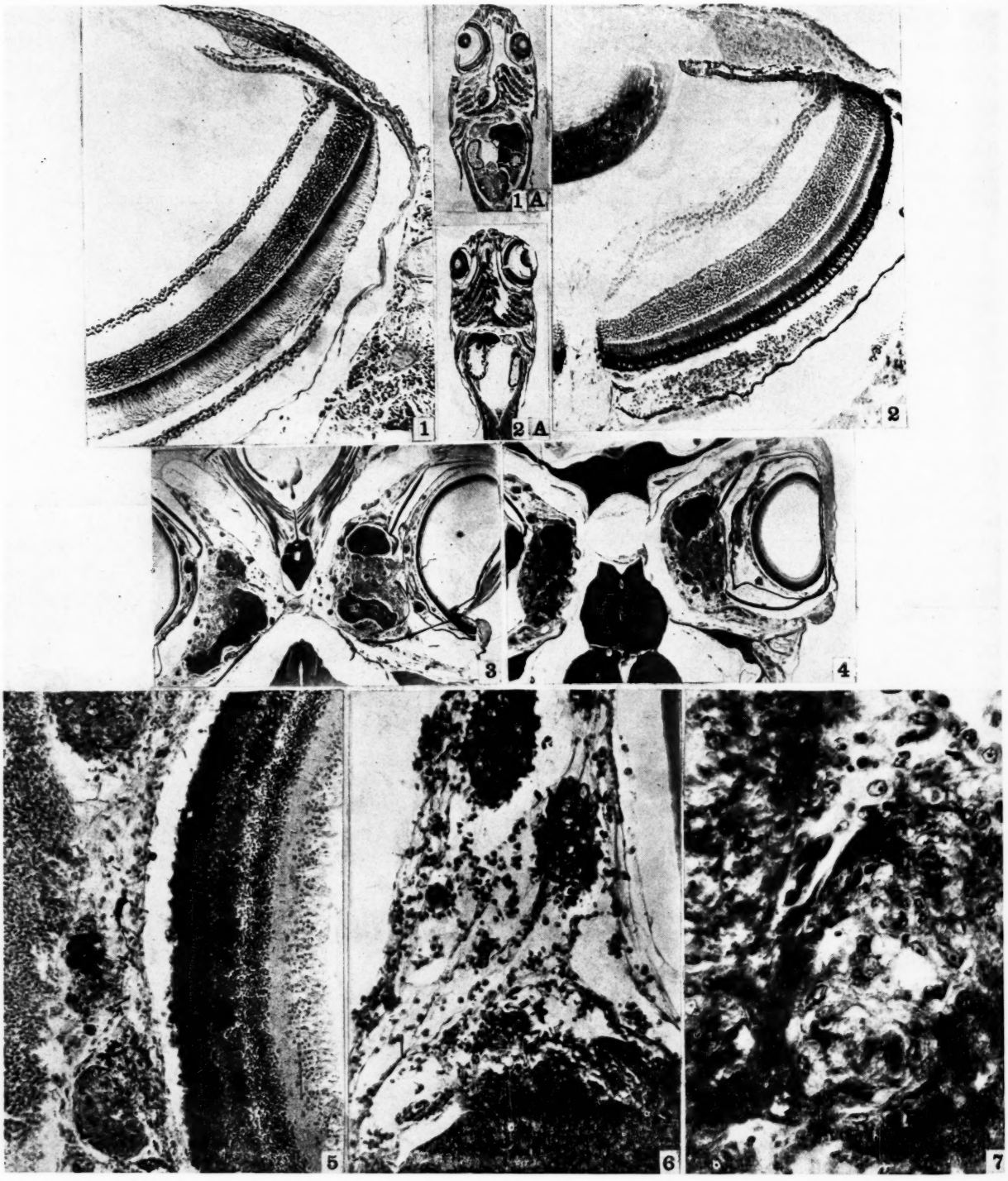

FIG. 1.—Longitudinal section of young normal albino hybrid, *X. hellerii* × *P. maculatus*. Mag. × 6.7.
FIG. 1A.—Radial section of eye. Mag. × 103.
FIG. 2.—Longitudinal section of young gray hybrid. Mag. × 6.7.
FIG. 2A.—Radial section of temporal part of eye. Mag. × 103.
FIG. 3.—*P. maculatus*, showing tumorous masses outside choroid but joined to it by a narrow bridge of neoplastic tissue. Mag. × 36.
FIG. 4.—As in Fig. 3, section toward ventral surface of head. Mag. × 103.
FIG. 5.—Section of retina with neoplastic masses in choroid. Mag. × 103.
FIG. 6.—Globular masses of epithelioid cells surrounded by fibroblast-like cells. Mag. × 103.
FIG. 7.—Epithelioid cells forming pearl-like bodies, or fascicles. Mag. × 432.

uniformly globular than those seen in the tumor of *P. maculatus* (Fig. 7). The nuclei of the spindle cells were not well differentiated, but the nucleoli stood out clearly as ruby-red bodies with Fleming's triple stain; the cytoplasm was contracted and formed tenuous threads at the poles of the cell.

This growth appeared to be composed of two types of cells, the spindle-shaped cells predominating. The possibility that the epithelioid cells may have been transformed into spindle cells deserves some consideration. The origin of the tumor is not clear, but the suggestion that it may have been a melanoma with its primary site in the uveal area is tenable, because of the unusual mitotic activity in this zone and the absence of epithelioid cells in the region of the optic nerve. Intercalary growth has not been reported in tumors, yet no division figures were observed in the spindle-cell portion of the growth. The structure of the neoplasm was suggestive of a neurofibroma. Ewing (3) points out that intracranial tumors of the nerves in man affect chiefly the acoustic nerve. In this fish, however, the optic nerve was somewhat infiltrated by small strands of melanin, but no more extensively than in normal fishes. It is quite likely that this is another phase of the choroid melanotic tumors seemingly characteristic of these fishes.

Platyfish-swordtail hybrid "A."—This hybrid had a glistening mass of hyaline tissue set out well above the normal contour of the head (Fig. 12). While the extraocular tumor made its appearance definitely in the choroid of the right eye (Fig. 13), it produced a notable exophthalmia and spread through the orbital space, especially toward the temporal region. The globe was displaced and rotated, producing an abnormally large anterior chamber. The tumor invaded and destroyed part of the retina and caused degenerative changes in the lens.

The inner surface of the intraorbital part of the growth consisted of a mass of red blood cells traversed by strands of fibrous material. The main tumor was continuous with tumorous tissue lying beyond the sclera (Fig. 13); the neoplasm and the invaded retina are shown under higher magnification in Fig. 14. The temporal side of the retinal layers was disorganized and invaded by epithelioid cells, and the tumor below showed the same characteristic cellular structure (Fig. 15). Melanin granules were densely scattered through the tissue. The fascicles of tumor were separated in the gross, perhaps because of some defect in fixation. The epithelioid cells were in active division. No spindle-like cells were in evidence. The organization and cellular composition of the tumor were identical with those found in the isolated uveal tumor in *X. pygmaeus* (Fig. 11). Here the evidence appears to indicate the presence of a melanotic tumor of choroid origin, with invasive characteristics.

Platyfish-swordtail hybrid "B."—This fish had a tumor of the right eye, with considerable exophthalmia (Figs. 16, 17). The orbital space was filled with a thick layer of tumor tissue that enveloped the inner surface of the eye, occupying the choroid layer and covering part of the cornea. The pressure induced distortion of the eyeball and its displacement upward and outward.

Here the retinal layers appeared to be thrown into folds, coupled with degeneration of the lens (Fig. 16). A tangential section through the eye at the level of the choroid revealed a mass of homogeneous neoplastic tissue (Fig. 17) whose upper portion contained what appeared to be a portion of the displaced optic nerve. Under high magnification the growth proved to be a uniformly compact mass of epithelioid cells, with some slight invasion of the optic nerve (Fig. 18). Its cells were similar to the epithelioid elements previously described in the ocular neoplasms. Here, again, the point of origin appeared to be the choroid, and the tumor seemed to be melanotic in nature. Both hybrids "A" and "B" were studied microscopically in their entirety, but no metastases were observed.

The fact that ocular tumors in these two hybrids appeared among the offspring of males of the same family may have some genetic significance.

Platyfish-swordtail hybrid "C."—This albinistic hybrid displayed an amelanotic melanoma in addition to unilateral exophthalmia. Sections made parallel with the surface of the head through the axis of the left eye showed an extruded, globular eye with its retinal layers and nerve forming a goblet-shaped structure (Figs. 19, 20). The anterior chamber of the eye was filled with a granular substance, while the posterior chamber contained the retinal layers, which were thrown into aberrant folds and separated from one another. The choroid and scleral tissues assumed a globular form, yet appeared to be much thicker than those of a normal eye. The optic disc (Fig. 21) represented the fundal portion, of conical, goblet-shaped structure. It was infiltrated by numerous epithelioid cells and melanin granules. The retinal layers were invaded by epithelioid cells that formed a loose network (Figs. 22, 23), and the spaces between the layers were filled with a serous fluid in which fibrillar bodies were seen. The nuclei of the epithelioid cells were well-differentiated (Fig. 24), and mitotic figures not infrequent. Large cells of the epithelioid type also made their appearance in these spaces, but were less abundant. This tissue seemed to be supported by a fibrous stroma. The spaces about the eye were filled with a reticulum of fibrous elements, in which epithelioid cells and granules were

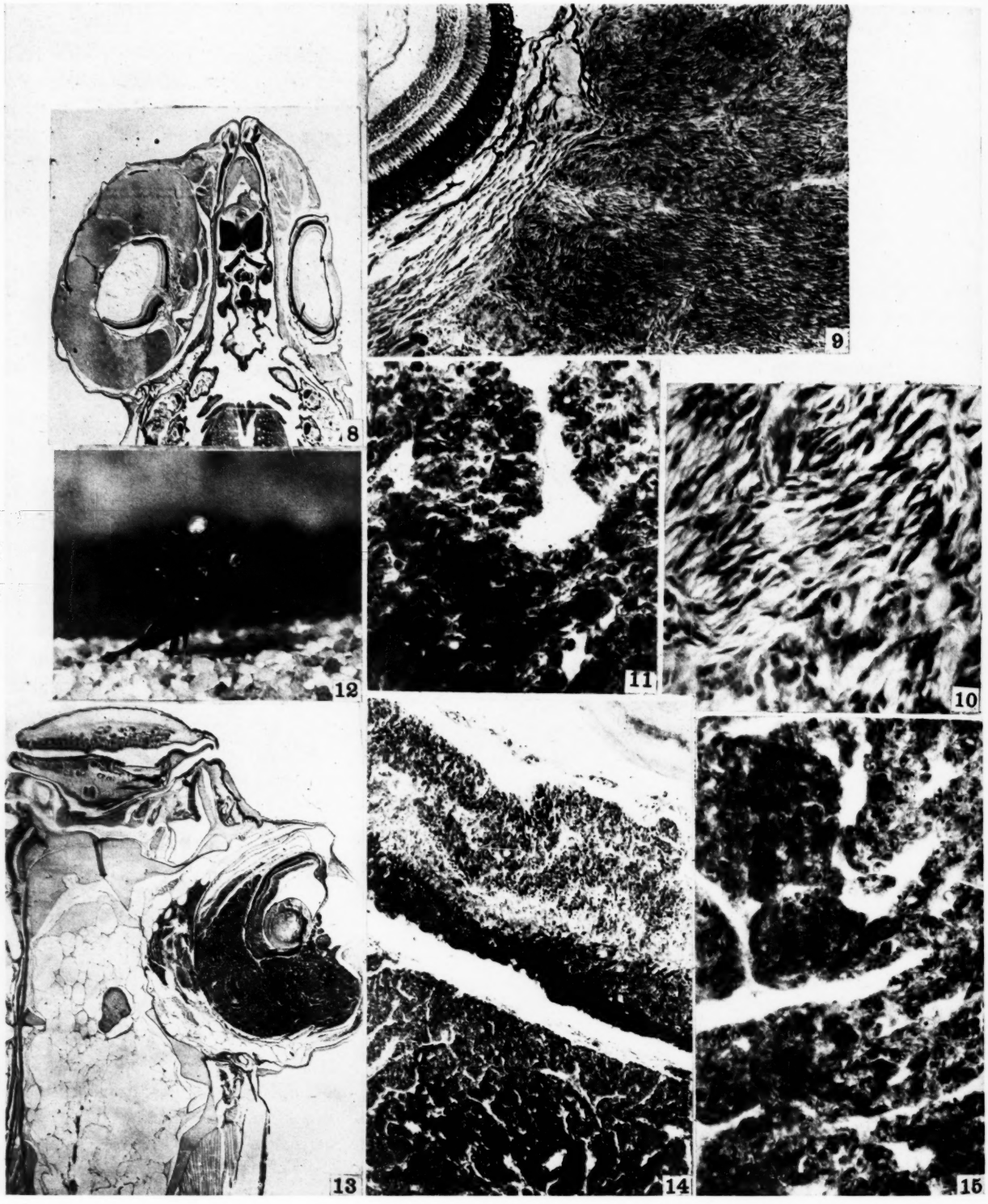

Fig. 8.—Longitudinal section of head of *X. pygmaeus* with tumor surrounding the right eye. Mag. × 36.

Fig. 9.—Section through tumor, showing part of retina, choroid, and tumor. Mag. × 103.

Fig. 10.—Spindle cell tumor, portion of Fig. 9. Mag. × 378.

Fig. 11.—Epithelioid cell from uveal area of same tumor. Mag. × 378.

Fig. 12.—*X. hellerii* × *P. maculatus* hybrid "A" (133 × 127-11), showing tumor protruding from orbit of right eye. Mag. × 4.5.

Fig. 13.—Ocular tumor of hybrid "A." Mag. × 36.

Fig. 14.—Portion of retina and tumor as in Fig. 13. Mag. × 103.

Fig. 15.—Epithelioid tumor cells as in Fig. 14. Mag. × 432.

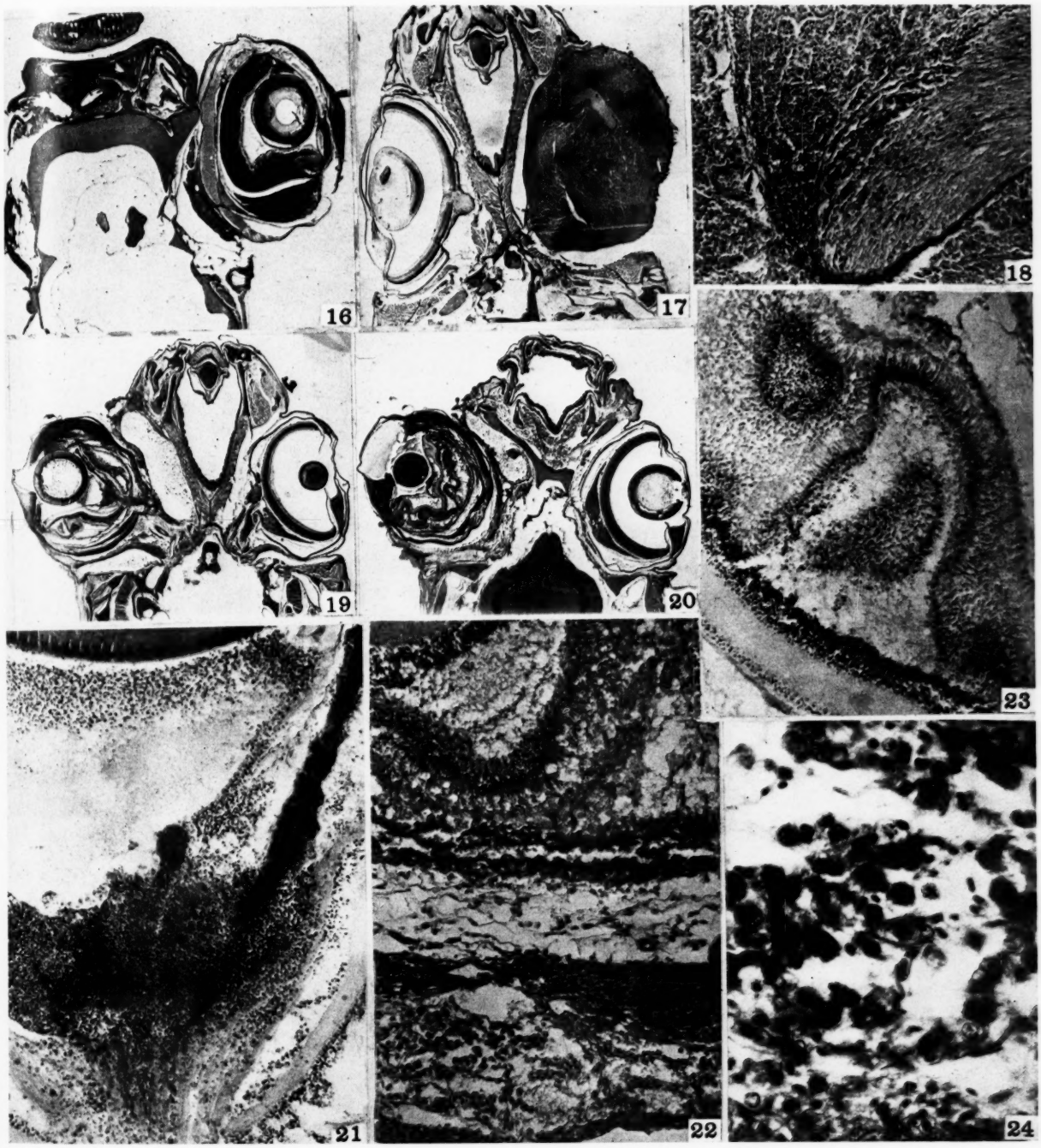

FIG. 16.—Ocular tumor of hybrid "B" (127 × 127-21). Mag. × 36.

FIG. 17.—Tangential section of eye, showing choroid tumor enveloping optic nerve of hybrid "B." Mag. × 36.

FIG. 18.—Section through tumor, showing portion of optic nerve. Mag. × 103.

FIG. 19.—Median section through left eye of amelanotic melanoma, hybrid fish, "C," showing goblet-like structure of inner layers of retina. Mag. × 36.

FIG. 20.—Another section through eye, showing folds of retinal layers. Mag. × 36.

FIG. 21.—Optic disc and portion of lens of same eye. Mag. × 103.

FIG. 22.—Retinal layers and choroid of left eye. Mag. × 103.

FIG. 23.—Retinal layer showing considerable folding, with edema and hemorrhage. Mag. × 103.

FIG. 24.—Epithelioid cells and red blood corpuscles comprising tumor tissue. Mag. × 378.

present. Blood cells were commonly found scattered among the normal cells. The right eye appeared normal, though the fundal part of the choroid was much enlarged, and consisted of numerous epithelioid cells.

That this ocular condition may have been due to pressure originating in the choroid was suggested by the folds of the retinal layer; it was obviously secondary, and induced by a choroid tumor that had given rise to edema and hemorrhage: its analogy to lesions of the human eye has not been determined.

The Etiological and Clinical Influence

The causative agent for these ocular tumors is unknown. There was no definite evidence of genetic influence, for many of the exophthalmic fishes represented single individuals in an otherwise normal brood. The hybrids "A" and "B," however, were genetically related, since their male parents were brothers (127-11 and 127-12). One of the males was mated to its sister (127 × 127-11), while the other was mated to an albino *Xiphophorus hellerii* (133 × 127-12). As these two broods were reared in aquaria that were kept side by side it was possible that the water in them may have become mutually contaminated; but when a large number of platyfish (*P. maculatus*) from an entirely different culture (163) were introduced into one of the tanks containing the sick fish, none of them or their offspring (163^2), which were born and reared together, showed the slightest indication of exophthalmia after a stay of 8 months. This seems to eliminate the possibility of an exogenous agent, unless it requires the same genetically constituted fish upon which to act.

The only support for genetic influences in exophthalmia rests upon the fact that more than the usual number of fishes developed ocular tumors in the two broods where one parent of each belonged to culture 127. The parents and siblings of this culture had normal eyes, and the entire brood consisted of over 100 specimens that reached the age of a year or more. If a genetic influence for ocular tumor was present, it cannot be expressed in simple Mendelian terms.

SUMMARY AND CONCLUSION

1. Among *xiphophorin* fishes, *P. maculatus, X. pygmaeus,* and hybrids between *P. maculatus* and *X. hellerii,* there were sporadic cases of exophthalmia due to melanomas of unknown origin.

2. Examination of these tumors revealed involvement of the choroid, with invasion of the retina and, finally, of the intraocular spaces.

3. These growths were comparable to melanomas of the human eye.

4. Their cells were predominantly epithelioid, but in one instance epithelioid and spindle cells appeared in the same tumor.

5. The cause of the tumors, like that of most animal neoplasms, is unknown. It was not any known microbic or viral agent, nor was it apparently a result of specific hybridizations or controlled by genetic factors. The growths cannot be produced at will, as can generalized integumentary melanomas in platyfish-swordtail hybrids (4), or certain tumors of plant hybrids (9).

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The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William B. Coley, M.D., Reviewed in the Light of Modern Research

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During the past six years an exhaustive study has been made of the treatment of malignant tumors by injections of bacterial toxins, as developed by the late Dr. Coley and other investigators. The problem has been approached in the following manner: (a) Is there sufficient clinical and experimental evidence to justify the conclusion that the method has therapeutic value? (b) If so, what factors governed success or failure, and why did the method not achieve recognition? (c) If the conclusions to the above questions warrant further study, what can be done to make the toxins consistently effective?

As there is no comprehensive monograph on the subject it was necessary to cover the literature thoroughly, analyzing in detail not only the records of Coley's own cases but those of the other investigators; this entailed extensive correspondence with hospitals, clinics, and physicians here and abroad who have used the toxins. It was considered necessary to study the records of cases in which intercurrent bacterial infections were said to have elicited profound effects upon malignant tumors. The experimental work on malignant tumors in animals has also been reviewed.

The first significant observation to be brought out was that at least fifteen different preparations of Coley toxins have been used since the method was introduced in 1892, of which three were considerably more potent than the rest. It was further observed that the technic of administration has varied considerably as regards site, dosage, frequency, and duration of treatment.

Unfortunately, the only preparation available in the United States since 1921 has been very weak, and the method employed in this later period has been for the most part much less effective than that used in the early years. Therefore, most of the present generation of physicians have not seen the remarkable results originally obtained.

As for clinical studies of the 15 different preparations, a number of physicians in this country and abroad, as well as Coley himself, reported cases successfully treated between 1895 and 1944. Among these were: Johnson (42), Owens (54), Mynter (52), McArthur (49), Stone (72), Battle (5), Fowler (31), Tupper (76), Walton (78), Thomas (73), Winberg (79), Lilienthal (46, 47), Green (34), Spencer (69), Tosier (74), Hertel (39), Greenwood (35), Harmer (36), Lagueux (44), Christian and Palmer (14), Blum and Coley (9), and DaCosta (25).

Others reported failures, and although their experience was based on a limited number of cases the majority seemed to consider it sufficient evidence to condemn the method unequivocally, apparently without considering whether the preparations or the technic employed might not have been at fault. Examples of these are: Emmerich (29), Campanini (13), Sheild (66), Newcomet (53), Babcock and Pfahler (4), and Senn (64).

Except for a few of Coley's longer papers and those of Moullin (51), Fowler (31), DaCosta (25, page 293), and Palmer (55), the method of administration does not appear in detail. However, Harmer described his experience during the period from about 1908 to 1914 (36).

It would appear that few except Coley, here or abroad, considered the possibility that the toxins might be expected to produce a larger number of permanent results in the earlier stages of the disease. An exception was Matagne, who wrote Coley in 1913 that for the previous 18 years he had consistently used the toxins before operation, and that this procedure had given him "a percentage of cures much higher than ordinary surgical treatment alone" (48).

Apparently no one physician or clinic treated a sufficient number of cases of each type of tumor in each stage of the disease to yield adequate material for a comprehensive analysis. It seemed necessary, therefore, to assemble detailed histories of a large number of cases of every type of tumor that has been treated by this method, and to study carefully the technic of administration. Over 600 histories have been abstracted in detail on the same form, covering the period since 1892; approximately 48 per cent were from Coley's cases. Also included are 65 cases in which

an intercurrent infection, principally erysipelas, caused regression in malignant tumors of various types.

In over 88 per cent of the cases studied the diagnoses were based on reliable microscopical, as well as clinical or roentgenological, findings. The cases were listed according to the type and site of the growth. It was found that the toxins alone or in combination with other methods of treatment had been used in the following groups of tumors in which 5 year survivals were obtained:

It has been possible to abstract 96 histories of carcinoma of various organs in which the toxins were used, as well as 21 cases in which an intercurrent infection occurred. A study of these histories, together with the experimental work of Shwartzman and other recent investigators, indicated the need for a more specialized technic of administration in cases of carcinoma and certain more slowly growing tumors (68); this was not known prior to Coley's death, in 1936. Experiment has demonstrated the presence of factors

End Results of 484 Cases of Malignant Disease with Histologic Confirmation in Which Coley's Toxins Were Used

Type of tumor	Total no. cases	Inoperable		Operable	
		Total	Five year survivals	Total	Five year survivals
Carcinoma	69	45	15	24	21
Malignant melanoma	24	19	4	5	3
Bone sarcoma	205	98	37	107	51
Soft parts sarcoma	123	91	53	32	25
Lymphosarcoma	49	45	24	4	4
Hodgkin's Disease	14	14	1	0	0
Total	484	312	134	172	105

In compiling this table the following cases were excluded: a) 80 that lacked definite histologic confirmation; b) 55 cases of giant cell tumor because of the difficulty in determining which of these were malignant; c) 32 cases in which complete regression occurred but which were followed less than five years.

Of a total of 312 inoperable cases there were 190 complete regressions. Of the total of 239 five year survivals, 101 were treated by Coley and 139 by other physicians. It should be noted that 15 of the five year survivals subsequently died of the disease, 8 of which were inoperable and 7 operable, when toxin therapy was instituted.

Concerning the use of the toxins in carcinoma, Coley stated that until he began using the more potent Buxton preparation in June, 1894, he had seen only temporary improvement in carcinoma. Within the next few months, however, he (17) saw 2 epitheliomas disappear entirely under treatment with the Buxton preparation (Type VI). Unfortunately, other physicians were using less potent products and less effective technic and were meeting with little or no success in treating carcinoma during those first years.

In 1897 Coley began to concentrate his attention on sarcoma because it seemed that the method was more effective here than in carcinoma; hence comparatively few carcinomas appear to have been treated during his lifetime by toxin therapy. But about 1930 he became aware that the results justified a change of attitude, for he stated: "During the first few years, I treated a considerable number of cases of inoperable carcinoma. . . . . While in most cases a certain amount of improvement was apparent . . . ., in the great majority . . . . [it] was . . . . only temporary and I decided to restrict the method chiefly to inoperable sarcoma. Later on, the number of cases of inoperable carcinoma apparently cured by the toxin treatment administered by other surgeons led me to the belief that I had greatly underestimated the value of the toxins in these cases" (24).

in some bacterial toxins that are destructive to tumor cells, but it appears that in order to kill the less vulnerable cells it may be necessary to sensitize them by injection into the growth or its immediate periphery, these sensitizing injections being alternated daily or every other day with intravenous injections. The results obtained in a case of carcinoma, generalized in the abdominal cavity and treated by intraperitoneal injections, suggest that the intraperitoneal route should receive further investigation in such cases. From a study of the clinical histories it appears that a longer period of treatment is necessary in the more slowly growing, less sensitive tumors in order to produce complete and permanent regression.

HISTORICAL OUTLINE

The treatment of malignant tumors by bacterial toxins is based on the fact that neoplasms of practically all types have been known to regress under acute bacterial infections, principally erysipelas (15). In many cases the inhibitory action was sufficiently powerful to cause complete disappearance of the tumor, and a number remained free from recurrence.

Coley's interest in the phenomenon was aroused by a thrice recurrent inoperable lymphosarcoma of the neck that had recovered under an attack of erysipelas (15). This led him to make a careful study of

the literature, where he found reports of 38 cases of inoperable malignant tumors in which an attack of erysipelas had occurred, either by accident or inoculation. Of these 17 were carcinomas, with 3 permanent regressions, and 17 were sarcomas, of which 7 did not recur. The other 10 sarcoma cases all showed distinct improvement, and in some of them the tumor disappeared completely but later recurred.

Beginning in April, 1891, Coley attempted to produce erysipelas as a therapeutic measure in 10 patients with inoperable malignant tumors. The difficulty in producing an attack and the dangers incident to inoculation with living cultures induced him to seek some method of eliciting the beneficial action of *S. erysipelatis* without the attendant risks. He therefore tried cultures sterilized by heating (at first to 100° C., later to 58° C.) or filtration, but all were found to be too weak to be effective.

In December, 1892, he learned of the investigations of Roger on *B. prodigiosus* (*Serratia marcescens*) in association with other organisms (60); in this connection are to be noted also the work of Vaillard, Rouget, and Roux (77, 61, 62). As these experiments suggested that *B. prodigiosus* or its toxins increase the virulence of other organisms with which they are associated in their proliferating stage they were incorporated in the formula that became known as Coley's toxins. Research by Beebe and Tracy (6, 75) and later investigations by Shwartzman (67) have clearly indicated that the toxins of *B. prodigiosus* are more potent in destroying the tumor cell than are various types of streptococci.

The first mixed toxins of these two organisms were prepared for Coley by Alexander Lambert at the College of Physicians and Surgeons, in New York. Cultures were sterilized by passage through a Kitasato filter, and mixed only at the time of use; these are called Type IV in this study. Although a number of tumors never recurred after treatment with this preparation, it appeared to be comparatively weak and distinctly variable.

Early in 1894 Buxton undertook to prepare toxins for Coley, and introduced a modification that consisted of growing the two cultures together. This preparation, also filtered, is called Type V. It appeared to be more potent than Type IV.

In June, 1894, Buxton prepared the first mixed, unfiltered toxins. Cultures were grown together, as in Type V, but were sterilized by heating at 50° to 60° C. The first erysipelas cultures were obtained from a fatal case. After 1894 virulence was maintained by frequent passage through rabbits (12). During 1900 a double sterilization was considered necessary (55). Type VI, made by Buxton from June, 1894, until 1906, appeared to be more potent than any other preparation of the same period; however, its variability appeared to increase and its potency to diminish somewhat during the period from 1900 to 1906.

Some time in 1895 the Lister Institute of Preventive Medicine undertook to prepare the Coley toxins for England. Little is known of the formulas used, but they are believed to have been Buxton's V and VI. From the scant number of cases in which these early English preparations were used, it appears that they were somewhat less effective than Buxton's Type VI. A serious handicap of the Lister Institute preparations was that apparently no printed directions or indications for use were enclosed with them, so that unless physicians communicated directly with Coley, or had access to some of his published papers, they had nothing to guide them (63).

Seven years after Coley introduced toxin therapy two important studies were made of this method. The first was that of Moullin (51), who stated in outlining the evolution of the method: "It has been known for many years, according to Fehleisen since the seventeenth century, that not only maligant growths, but chronic ulcers of the skin, lupus nodules, syphilitic sores and other affections, occasionally disappear with great rapidity after an attack of erysipelas. The number of cases that have been recorded with sufficient accuracy and detail is not a very large one it is true. They are undoubtedly of exceptional occurrence. Many of them are of somewhat ancient date, as might be expected from the fact that erysipelas is much less common now than it used to be, and that attacks are less severe than in pre-antiseptic days, but after making full allowance for all these defects, there still remains a sufficient number of well-authenticated instances to dispel at once the idea that the disappearance can in any way be due to mere coincidence.

". . . . Such cases as these—isolated though they were—could not fail to attract attention, and even before the microbic origin of erysipelas was known, several attempts were made at inducing an attack by means of inoculation. When Fehleisen discovered the streptococcus, and it was shown that pure cultures could be obtained, it was naturally not long before a systematic attempt was made. Fehleisen himself was the first.

". . . . Coley and others quickly followed suit, but two things very soon became apparent. First, that it was exceedingly difficult in many instances to induce an attack. . . . . Secondly, that it was still more difficult to limit the effect of an attack when it did occur. There are 25 cases recorded in which an attempt was made to induce an attack of erysipelas by inoculation. In nearly all, pure cultures were injected or rubbed in after the skin had been scarified; in one the patient was placed in a bed which had a notoriously bad

history. Six never had a genuine attack. . . . . Four of the remaining nineteen died as a result of the attack.

"Fourteen of these cases were definitely sarcomata. One of them under the care of Dr. Coley, a sarcoma of the neck that had already recurred twice, was cured; that is to say four and a half years afterwards the patient was well in every respect. Another, a lymphosarcoma of the neck which had resisted arsenic, under the care of Kleeblatt, disappeared entirely. . . . . In two others, under the care of Coley, after repeated inoculations with living cultures, each of which was followed by temporary improvement, a cure resulted from the use of the mixed toxins. . . . . Two others, an enormous sarcoma of the neck . . . . and of the tonsil . . . . almost disappeared, but as soon as the effects of the erysipelas had passed off, began to grow again. Two of Fehleisen's diminished considerably for a time. Four were scarcely affected, and in the remaining two erysipelas never occurred at all.

"It is worth noting that in every single case in which erysipelas occurred the tumors showed some change, although in four of the twelve it was very slight." [1]

Moullin describes the effects produced by the toxins on malignant tumors as follows: "When there is an ulcer or fungating sore, inflammation and sloughing appear to be the rule. In one or two instances the whole tumor has been thrown off in this way. On the other hand, with an unbroken surface, sloughing is the exception. . . . . The difference seems to depend upon whether pyogenic organisms are present and can gain access through some accidental abrasion. If they are present the tumor sloughs, if they are not it undergoes a process which for rapidity and thoroughness can only be compared to acute yellow atrophy of the liver" (51, page 21).

As to the microscopic changes produced, Moullin stated: ". . . . The only definite description that I can find is in the accounts of three cases, one of sarcoma, two of carcinoma, in which the tumors were . . . . disappearing during an attack of erysipelas when it proved fatal. . . . .

"One of these was a round-celled sarcoma of the neck, recorded by Busch. . . . . The sarcoma cells had undergone fatty degeneration . . . . around its outskirts, portions of the growth that were still unaffected allowed its character and the nature of the changes it was undergoing at the time of death to be ascertained (11, page 245).

"Very much the same appearances, making allowances for the difference in structure, was presented in two cases of carcinoma. In Neelsen's . . . . the contents of the alveoli had undergone complete fatty degeneration. . . . . Fatty degeneration was also found by Spronck in . . . . tumors removed from dogs . . . . under treatment with . . . . erysipelas toxins; and on several occasions in which tumors have been incised under similar conditions large quantities of yellowish-white fluid, with numberless fatty granules suspended in it, have been evacuated" (51).

It is interesting to analyze the type of investigation that helped to throw doubt on the effectiveness of bacterial toxins. To quote Moullin: "Friedrich, for example, after trying the toxins (of erysipelas alone) on nineteen cases, came to the conclusion that they had no specific effect of any kind, and that the changes the tumors sometimes undergo are 'merely the expression of the cumulative action of a number of injurious influences working together.' Of the nineteen cases he records, however, four only were sarcomata and two lymphosarcoma. The rest were carcinoma. . . . . Moreover, though the streptococci were taken from many different cases, it is by no means certain that they were sufficiently virulent. . . . ."

Moullin's conclusions as to the value of toxin therapy are: (a) That a considerable number of hopelessly inoperable sarcomas, many of which were recurrent, have completely regressed under this treatment, and that there is no other of which this can be said. (b) That the disappearance of sarcoma is not due to inflammation, but to an intensely rapid form of fatty degeneration comparable only to that which affects the hepatic cells in acute yellow atrophy of the liver. Inflammation and sloughing, when they do occur, are septic complications. (c) That degeneration or absorption may occur whether the toxins are injected directly into the tumor or at a distance. (d) That the toxins are of no value unless derived from a virulent case of erysipelas, or are made virulent by passing the streptococcus through rabbits. (e) That *B. prodigiosus* immensely increases the reaction. (f) That the effect is most striking in rapidly growing sarcomas; more slowly growing ones appear to have much more resistance. (g) That patients often gain in weight and strength while under treatment. (h) That the toxins may not be so effective against a recurrence. (i) That the severity of the reaction is variable, apparently depending upon the rapidity with which the toxins are absorbed. (j) That disappearance of the growths is not the result of high temperature alone, since fever from other causes is not followed by this result (51).

At about the same time Coley, too, described the macroscopic changes following treatment. "The toxins produce marked decrease in vascularity. . . . . The

[1] The experiences cited above appear to indicate that permanent results occur rather rarely following the brief toxic effect of an attack of erysipelas or some other acute bacterial infection. Apparently a more sustained toxic action, or a larger quantity of toxins than is usually generated during a single attack of erysipelas, is necessary to destroy the tumor completely and prevent recurrence.

tumor soon becomes much more mobile and . . . . decrease in size is often noticed within two or three days after the treatment is begun.[2] Cessation of pain is in many cases caused by the treatment, and anodynes, though indispensable before, are no longer needed." This beneficial effect upon pain had already been noted by Finney (17, page 161) and later was observed by a considerable number of other physicians. Among these was Lagueux, who, in reporting cases of successfully treated carcinoma, said that the pain entirely disappeared almost immediately after the first injection and that it was not necessary to lose time by giving small doses; the more quickly fever was induced the more often was success achieved. He added that while he did not wish to seem more enthusiastic than Coley himself, he believed that at least 60 per cent of inoperable sarcomas and carcinomas could be successfully treated (44, page 470).

The second comprehensive study was that of Fowler (31). After reviewing the literature rather thoroughly, including the effects of intercurrent infections on malignant disease as recorded by Fehleisen (30), Busch (11), Billroth (8), Biedert (7), Plenio (56), Bruns (10), Coley (15), Mishtolt (50), Stein (71), Kleeblatt (43), and Wyeth (80); as well as the attempts to produce erysipelas in cases of malignancy recorded by Répin (57), Holst (40), Janicke (41), Lassar (45), and Spronck (70), he described the evolution of Coley's final product, the mixed toxins of erysipelas and *B. prodigiosus*.

Fowler called attention to the varying effects that followed injection at different sites in Répin's experience. Répin used the subcutaneous route at first, but because of the irritation and edema changed to intravenous injection, which, he found, caused no local disturbance and at the same time elicited a more prompt, decided, and uniform reaction. Fowler stated that the intensity of the general reaction varied with the dosage and method of administration; when the injection was made subcutaneously a larger quantity was required to produce the desired reaction, whereas a few drops were sufficient when the intravenous route was used. He added: "The vascularity of these tumors explains the ease with which a reaction can be produced by Coley's method of interstitial injections, the latter being quite analogous, if not identical with, the intravenous method." In Fowler's experience a chill, the first noticeable symptom, occurred from 15 minutes to 2 hours after injection and rarely lasted longer than 10 minutes. In further describing the effects of the injections Fowler said:

"The symptom of elevation of temperature likewise depended on the dose. This followed the chill after three to six hours, if the latter occurred, and in some instances reached 40° C. The fever lasted from eight to ten hours. It was accompanied by the usual correspondingly increased pulse rate and frequency of respiration.

"Cephalalgia sometimes occurred, as well as nausea and slight vertigo. Herpes labialis, sweating of the palms of the hands, and fugacious eruptions were observed in some cases in which the fever was well marked. Estimates of the amount of urea showed this at times to be somewhat lessened, albumin was not found at any period.

"Examinations of the blood made ten minutes after the intravenous injections showed slight diminution in the number of leucocytes present. The subcutaneous injections were followed by no change in this respect. Leucocytosis was occasionally observed, this being more noticeable after the intravenous injections.

"Emaciation supervened rapidly with the use of large doses. . . . . This, according to Répin, need excite no alarm, and should not constitute sufficient justification for discontinuing the treatment."

It should be noted that a study of several hundred cases with various types of tumors indicates that emaciation occurs only where the neoplasm is extensive and large quantities of necrotic tumor are being absorbed under toxin therapy. These degenerative products appear to be hemolytic, and where tumors break down rapidly their absorption may cause toxemia or cachexia, which disappears when free drainage is established or the tissue has been absorbed.

As regards Coley's technic of administration prior to 1898, Fowler stated: "All of Coley's patients have been treated by interstitial injection into the tumor itself. This circumstance rather enhances the value of the evidence. . . . . While it is true that decided effects can be produced by irritating substances . . . . such . . . . as turpentine . . . . these are not of curative value; furthermore, they have no elective action when injected at a distance. . . . . Once the specific action of the toxins is admitted, it is highly probably that this should be exerted more energetically in situ than when injected at a distance."

Not until after Coley's death, in 1936, did other investigators demonstrate that to be most effective the toxins must reach the tumor via the blood stream as rapidly as possible, before they are neutralized by the tissues (67). This would indicate why intratumoral and intravenous injections produced more rapid destruction of the growths. Unfortunately, however, Coley gradually ceased using intratumoral injections in the period between about 1912 and 1915. He did not begin using intravenous injections until about 1926 to 1928. This route, tried at the suggestion of B. L. Coley, proved considerably more effective than intra-

[2] This did not apply to bone tumors, which respond more slowly.

muscular injections and more easily tolerated by the patient.

Fowler's appreciation of the importance of producing definite reactions should be emphasized. He wrote: "While it is true that the more decided the reaction as shown by the temperature, the better the outlook for a favorable influence upon the disease, it is not desirable that this should exceed 103° to 104° F. This is surprisingly well borne." As to the frequency of injection he said: "In the absence of excessive reaction or great debility, the injections may be given daily, with the expectation of obtaining two or three well-marked reactions during the week. With the occurrence of marked diminution in the growth, frequency may be diminished." Fowler believed that the injections should be kept up 3 or 4 months, with occasional intervals of rest lasting 3 or 4 days.

Fowler stated that, unlike most therapeutic agents, the toxins varied greatly in their effects upon different patients; so much so that it became necessary to establish the dose in each case by commencing with the minimum amount that would produce an effect in those most susceptible. Thus safety was assured, although a greater amount of time was required.

In making the present study the data on the toxins used prior to Fowler's observation were thoroughly reviewed, and it became evident that the variable response may have been largely due to the use of at least 8 different preparations. Fowler was apparently aware of the danger that immunity to the toxins might develop, for he wrote: "There can be no question that the general effects of the toxins become rapidly less pronounced in the course of treatment as immunization becomes established." He added that whereas a comparatively small dose was sufficient to produce a decided rise in temperature early in the treatment, this had to be increased many times to obtain the same effect as treatment progressed. He further stated: "Inasmuch as the local reaction upon the tumor follows a course parallel with the intensity of the general reaction, it necessarily follows that as immunization becomes established the effect upon the growth becomes less and less until at last it becomes nil, and the neoplasm, released from the restraint exercised upon it by the toxins, resumes its former progressive march . . . ." (31).

In discussing the rationale of the action of bacterial toxins on neoplasms, Fowler discussed the theory that the occurrence of high temperatures exerted an influence on the vitality of the tumor cells: "It is difficult to understand why conditions unfavorable to cell life created by the fever of erysipelas should differ in this respect from those arising from the fever, the result of other diseases." It was not until over 30 years later that the exhaustive research of Shwartzman and several other investigators indicated that not all bacteria generate toxins capable of eliciting necrosis and regression in malignant tumors. However, some of these investigators, including Andervont (1) and Apitz (3), indicated that the destructive process may be enhanced by thermal hyperemia, apparently as a result of increased tissue permeability.

First Commercial Preparation

In October, 1899, Parke, Davis and Company undertook to produce the Coley toxins commercially, using Buxton's Type VI formula. This preparation, Parke, Davis Type IX, was made from late in 1899 until 1907 and letters found in Coley's files clearly indicate its comparative weakness and variability. One reason for its uncertain strength was that the streptococcus and prodigiosus cultures grew with variable luxuriance and no method had been devised to standardize the concentration of the suspensions. Cases in which both the Buxton Type VI and the Parke, Davis Type IX toxins were used further confirm the observations as to the comparative weakness of the latter. However, the technic of administration used by most physicians in this period was a favorable one; that is, injections were generally made into or near the tumor, and a certain number of successful results were obtained when the less potent Parke, Davis preparation was administered aggressively.

Huntington Research Fund Preparations (Tracy's X and XI)

Beebe and Tracy (6) appear to have done the first experimental work in this country on the effect of bacterial products on malignant animal tumors. They employed sterilized suspensions of four types of bacteria: *B. prodigiosus, Streptococcus pyogenes, Staphylococcus pyogenes aureus,* and *B. coli communis* (*Escherichia coli*). The streptococcus was from a fatal case of septicemia. The organisms were grown for 3 weeks in ordinary peptone broth; glycerine to the strength of 20 per cent was then added and a small piece of thymol as a preservative, and the suspension was heated to 75° C. for 1 hour. Later, in order to obtain a more potent preparation, the cultures were centrifugalized and the bacteria washed several times with sterile physiological saline, in a very little of which they were finally suspended. To this concentrated suspension glycerine and thymol were added as before, and the mixture was sterilized at 75° C.

The toxic properties of *B. prodigiosus* had received little attention since Roger's work (60), so a preliminary study was undertaken by Tracy. The organism was shown to contain highly toxic substances lethal to animals in very small amounts. Subcutaneous in-

oculation of a nonlethal dose of the suspension produced coagulation necrosis, while autolysis of the bacilli at 38° C. for 2 weeks set free agents that passed easily through a Berkefeld filter and produced toxic effects identical with those elicited by the suspension. These toxic substances could be separated into two distinct fractions, the alcohol-insoluble fraction being highly toxic, whereas the alcohol-soluble fraction was chiefly hemolytic.

As preparations of these two fractions failed to produce a local lesion comparable to that caused by suspensions of the whole organisms the authors felt that little effect upon tumors was to be expected, and experiment verified this conclusion.

They standardized their bacterial suspensions by determining the nitrogen content, expressing dosage in mgm. of nitrogen. In order to bring the preparation of the mixed toxins into line with this method of measurement, a definite quantity of prodigiosus suspension of determined nitrogen content was added to the broth culture of streptococcus so that each cc. of the mixed product contained 2.5 mgm. of prodigiosus nitrogen.

These preparations were all tried on dogs bearing large transplanted lymphosarcomas, with the following results: (a) Concentrated suspensions of streptococcus were necessary to produce effects. (b) Killed cultures of *B. prodigiosus* were definitely destructive to the tumor, as were the toxins of *B. coli.* (c) The toxins of *Staphylococcus pyogenes aureus* had no inhibiting influence, even though injected directly into the growths. Necrosis set in at once in tumors that had been directly injected and within 24 hours considerable discharge of necrotic tissue took place, so that by the second day only the smallest remnant of tumor was still palpable. On the other hand, no sign of regression occurred in a dog treated by intramuscular injections remote from the tumor until the end of the second week of treatment. The need for surgical drainage where fragments of necrotic tumor tissue are not absorbed was established.

In conclusion Tracy and Beebe stated: "The results . . . . certainly demonstrated the destructive action . . . . on tumor cells of this type by bacterial toxins. Such action, while chiefly local, is at the same time something more than this, for it is repeatedly observed that tumors at a distance . . . . undergo regression simultaneously with those inoculated, while in one instance the entire treatment was by inoculation remote from the tumor" (6).

As a result of these studies, Tracy evolved the first stable preparation of Coley's mixed toxins, and it proved to be considerably more potent than any other prepared from 1892 until 1942 (19, 20). A streptococcus culture from a fatal case of septicemia was used. The two organisms were grown separately and heated to 75° C. for an hour in a water bath. The prodigiosus was then reduced to a dry powder and the amount employed, determined by Kjeldahl's method, was 5 mgm. per cc. of the mixed toxins. After mixing and bottling the preparation was again sterilized in the water bath at 75° C. for 2 hours, and thymol and glycerine were added as preservatives. Coley found this product, Tracy's Type X, much more stable as well as more powerful in its action, and said that the results in inoperable sarcoma had shown a distinct improvement over those obtained with the older preparations (20). Type X was made from early in 1906 until the latter part of 1907, when the amount of prodigiosus was reduced to 2.5 mgm. per cc. of the mixed toxins (21, 22). This preparation, Type XI, was made by Tracy from the latter part of 1907 until June, 1920. From then until late in 1921 the same formula was prepared by Dr. Morton Kahn, with only the modification that he used the single cell method to insure purity of the cultures.

It appears that Coley recognized the importance of technic of administration at the time Tracy's preparations were introduced, for he said in 1909: "Much depends upon a judicious determination of the dosage for a given case. As a rule I give as much as the patient can safely stand. I always begin with ¼ minim, diluted in sufficient boiled water to insure accuracy, injected either in the buttocks or the pectoral region. After the individual's susceptibility has been ascertained, one can inject into the tumor itself if it is not in an inaccessible region. The initial dose into the tumor should always be less, not more than one-fourth that used elsewhere. [He advised alternating between the tumor and the muscle.] Daily injections should be given, increasing by one-fourth minim until the desired reaction, a temperature of 102°-104° is obtained. This should be modified to suit patients in a weakened condition. Having secured the desired reaction, the dose should no longer be increased until it fails to give a reaction. . . . . the highest dose given in many of the cured cases has been seven or eight minims." He usually preferred to give the injections locally, for he had found the effect more rapid and more definite than when given subcutaneously and remote from the tumor. "The dose depends largely upon the vascularity of the tumor and upon the condition of the patient" (22).

The highest number of successful results in various types of tumors was obtained in the period from 1906 to about 1912, when Tracy's potent and stable preparations were first being used, and when the effective technic described above was being advised.

It is evident, however, that Coley realized there were certain weak and unreliable preparations being dis-

tributed as "Coley's Fluid" prior to 1907, for after describing in detail the formulas used by Tracy he stated: "It is important . . . . to know how [the toxins] have been prepared, for the results vary greatly with their composition and manner of preparation. Many . . . . now on the market have been so weak as to produce hardly any reaction and have been found . . . . of little value. Parke, Davis and Company, we know, have made great efforts to keep their product up to the standards of the [Huntington] Research Laboratory; of other preparations I have no personal knowledge" (20).[3]

Late in 1907 or early in 1908 Parke, Davis and Company adopted the Tracy Type XI formula (designated as Parke, Davis XII). Unfortunately, the formula Tracy sent to Parke, Davis apparently did not specify at which stage the nitrogen determinations should be made or the need for using chromogenic cultures of *B. prodigiosus*. For these and other undetermined reasons the Parke, Davis preparations continued to be weaker than those of Tracy. This statement is based on the experience of a considerable number of physicians here and abroad, as well as with some of Coley's cases. While some of these men realized that something was wrong, a far larger number undoubtedly did not, condemning instead the fundamental principles of the treatment.

Finally, in April, 1915, a particularly clear-cut example of the comparative weakness of the Parke, Davis product was reported to Coley by Tuholske (23). At Coley's suggestion, Parke, Davis, in collaboration with Tracy, attempted to make their produce more effective. The resulting preparation was designated as Parke, Davis Type XIII. Since 1915 the following slight modifications have been made in this formula: (a) Some time between 1915 and 1922 the attempt to maintain virulence by passage through rabbits was abandoned. (b) In January, 1922, a new streptococcus culture was obtained from a case of erysipelas.

It should be noted that Tracy also prepared filtered toxins in the period during which the unfiltered Type XI was available (1906 to 1921), as did Parke, Davis. These appeared to be considerably less potent than the unfiltered toxins, although the formulas are believed to have differed only as regards the method of sterilization. It was observed in a number of cases in which both the filtered and the unfiltered toxins were used that the patients did not seem to develop immunity to the filtrate as they did to the unfiltered preparations. However, from the evidence available, it appears that the filtrates used by Coley and other physicians were not so effective.

The Lister Institute adopted Tracy's Type XI formula some time in 1907 or 1908, and has continued to make this preparation until the present time. However, the cases available for study are not sufficient to justify full comparison of its potency with that of Tracy's Type XI, or the Parke, Davis products available in the same period.

No experimental work seems to have been done in this field between 1907 and about 1930. Interest in the bacterial toxins was revived in 1931 by Gratia and Linz (33), who, having attempted to produce local tissue reactivity in a liposarcoma of the guinea pig, found the filtrates capable of eliciting the phenomenon, and also hemorrhagic necrosis, in the tumors without any preparatory injections.

Shwartzman and Michailovsky described the same phenomenon in a rather extensive series of mice bearing transplanted sarcomas (68). Duran-Reynals pointed out that besides their intrinsic value two considerations make these findings particularly interesting: (a) That mice are insusceptible to the ordinary Shwartzman phenomenon (local tissue reactivity), and it would seem that special conditions in the tumor render its vessels susceptible to the toxins. (b) That this state of reactivity in the tumor in either susceptible or nonsusceptible animals is a permanent one so that no sensitizing injection is required, as is the case with the ordinary Shwartzman phenomenon in rabbits and guinea pigs (26, 27). Another significant observation of Duran-Reynals was that animals in which tumors had regressed after injections of bacterial toxins were resistant to reinoculation. He reported on the vascular reactions to a *B. coli* toxin of low potency of a series of growths ranging from benign embryomas and granulomas to highly malignant transplantable carcinomas and sarcomas of mice and rats. He concluded that only those growths showing at the same time *malignancy* and *rapidity of growth* present the phenomenon described by Gratia and Linz. Duran-Reynals stated: "It is obvious that the phenomenon is conditioned by two sets of factors: (a) The intrinsic factor, depending on the sensitivity of the tumor itself. (b) The extrinsic one, depending on the activity, quantity and route of inoculation of the toxin." Additional studies showed that very susceptible tumors required smaller doses of toxin to produce the same effect, and the same result was obtained with filtrates of low toxicity as with the more active ones (26). Duran-Reynals also noted that the route of inoculation did not seem a matter of much importance in treating these rapidly growing tumors. In conclusion he stated: "The facts brought to light in transplanted as well as in spontaneous tumors establish the principle that the newly formed vessels of maligant growths . . . . are extremely sensitive to . . . . bacterial toxins, and . . . . this fact . . . . creates . . . . the tumor vulner-

[3] We have been unable to find any data on these.

ability . . . . responsible for . . . . regression . . . . (26, 27).

The exact nature of the effect of bacterial filtrates on transplanted tumors has not been elucidated. It is now known that it resembles closely, if it is not identical with, local tissue reactivity (Shwartzman's phenomenon) (67). The most striking macroscopic and microscopic finding is hemorrhage in the tumor about 4 hours after injection of the toxins. Apitz noted edema of the tumor cells, which he believed to be independent of the hemorrhage, for while both were produced by large doses, only edema was seen with smaller ones (3). Apitz observed no effect unless a lethal or sublethal dose was used—further evidence of the need for aggressive treatment in human patients.

Andervont found that the macroscopic reaction, a bluish discoloration within the tumor, that increased in intensity for several hours, was clearly visible 2 to 4 hours after injections (1). When complete destruction of the tumor occurred the discoloration continued until the growth became black, and within 24 hours all that remained was a hard, dry mass of dead tissue. This scab usually persisted for a week or two and then dropped off, exposing a mass of granulation tissue.

Microscopic studies of tumors removed at 2 hour intervals revealed an accumulation of white blood cells in the capillaries about 2 hours after injection. This was followed by congestion and eventual diffuse hemorrhage. Some of the tumor cells were swollen prior to hemorrhage, indicating that the toxins may have a direct effect. Andervont and Shimkin (1) believe that transplanted tumors regress after these injections only when gross hemorrhage is produced in the tumor, and that regression is directly proportional to the amount of hemorrhage; thus if only the center is affected growth of the nonhemorrhagic periphery is uninterrupted.

The need of early treatment is suggested by the work of Duran-Reynals, who stated: ". . . . mice having larger spontaneous tumors and receiving larger doses of toxins had a greater intensity of local reaction, a higher early mortality and a lower per cent of partial or total inhibition than . . . . the group with . . . . small tumors receiving smaller doses of the toxins" (27, 28).

Gardner, Bailey, and Hyde found that maximum hemorrhagic reactions after intravenous injection are determined by an optimal dosage, which causes a prolonged and intense systemic reaction and depends upon the sensitivity of the host. Reaction in the tumors was discernible 30 minutes after injection. Twenty-four hours later, sections showed definite hemorrhagic discoloration in those areas outlined by necrosis (32).

Perhaps the most persistent and exhaustive experimental studies in this field have been made by Shear, who has isolated an active fraction, apparently a polysaccharide, from the toxins of *B. prodigiosus* (*Serratia marcescens*) that appears to be 1,300 times more potent than the Parke, Davis Type XIII preparation (38, 65).

The Lankenau Hospital Research Institute recently began a study of the effects of Shear's polysaccharide upon human neoplasms, a significant feature of which is biopsy, not only before but during treatment.

DISCUSSION

The various preparations of Coley's toxins, and laboratory research in this field during the first 50 years after their introduction, has been reviewed in considerable detail because there has never been a comprehensive analysis of this method. The data indicate rather clearly the importance of maintaining closer cooperation between research and commercial laboratories and the physicians who administer toxin therapy.

A brief review of some of the other factors that hindered the acceptance and further development of toxin therapy may be of value. One of the first was the explanation that disappearance of a tumor under the treatment was probably due to spontaneous regression, and thus merely coincidental. These critics do not appear to have considered that the majority of so-called *spontaneous regressions* recorded in the literature occurred during or following an acute bacterial infection of some sort, including erysipelas, pneumonia, pyemia, typhoid, and others. Furthermore, the frequency with which neoplasms failed to recur after the administration of Coley's toxins precludes serious consideration of this criticism. Another objection most often heard was that the successful cases had not been unequivocally found malignant rather than benign tumors. Fortunately, Coley recognized the need for searching diagnostic tests in his cases, and the clinical and roentgenological data were supplemented by microscopic examinations by such leading pathologists as Ewing, Wolbach, Welch, Whitney, and Stewart, or the pathological departments of the larger hospitals. The majority of the bone cases were reviewed by the Bone Sarcoma Registry Committee.

A somewhat later criticism, namely, that if toxin therapy had the value claimed by Coley it should have become universally adopted was answered by Coley as follows: "I will call attention to one fact, apparent to anyone familiar with the history of medical discoveries; that the relative value of such discoveries bears not the slightest relation to the rapidity of acceptance by the medical profession" (24). We would add that even where scientific discoveries are accepted fairly soon, enormous difficulties have to be overcome before any new method becomes generally adopted.

This is particularly true in the field of cancer therapy, which for more than two thousand years has been ridden with quacks and so-called cures. In the case of toxin therapy the natural reluctance of physicians to embrace any radical new therapeutic measure was strengthened by the special problems already described: variability of the preparations, and ignorance of the optimum technic of administration. In addition, it is believed that the discovery of x-rays and radium so soon after toxin therapy was introduced may have played a part, for these commanded the attention of the profession everywhere, stimulating research and attracting munificent endowments. Thus radiology achieved popularity at a period in which toxin therapy had not yet been adequately developed.

Still another handicap was the lack of readily accessible literature. Coley's earlier papers and those of Fowler (31), Moullin (51), Harmer (37), Beebe and Tracy (6, 75), as well as the reports of successful cases treated by other surgeons here and abroad, are probably to be found only in the larger medical libraries, and considerable time and effort are required to locate and read them. Studied separately, they give only a fragmentary and rather confusing picture of what was accomplished.

Another factor that has received little or no consideration is the possible effects that other forms of treatment, given before or during toxin therapy, might have had upon the latter. In making the present analysis this was carefully studied. It was found that any agent that alters or destroys the vascular or lymphatic channels through which the toxins must reach the neoplasm or inhibits the regeneration of normal tissues, such as heavy radiation or repeated incomplete surgical procedures, appears to limit the effectiveness of subsequent toxin therapy. Since recent cytological studies indicate that the neoplastic cell is most responsive to toxin therapy during division, it would appear that anything that inhibits the rate of mitosis during toxin therapy may also slow up or minimize the destruction of tumor tissue by this treatment. These factors suggest that where the use of both toxins and radiation is contemplated, toxin therapy should invariably be completed before radiation is instituted. It has been further noted that any process that decreases tissue permeability may have a deleterious effect on toxin therapy. This point deserves further study with a view to using invasive strains of bacteria, or including a "spreading factor" such a hyaluronidase, as a means of possibly enhancing the effect of bacterial products (28).

To establish the treatment of malignant tumors by bacterial products on a more scientific basis, the following program is suggested:

1. Publish clinical data, to provide a comprehensive history and an analysis of the treatment. This would serve as a guide to the method, prevent some of the mistakes and misconceptions of the past from being repeated, and stimulate further research.

2. Encourage bacteriological research to make more potent and dependable preparations available. Many organisms are known to possess high titers of tumor-destructive toxins, and many more will need to be investigated. The ultimate goal is to isolate the active principles and produce stable and dependable preparations.

3. Institute clinical research on all types of tumors where the diagnosis is unequivocally established, using purified and concentrated preparations. Such methods as surface application of toxins to fungating tumors; the injection of minute doses into different parts of a tumor; continuous intravenous drip; hypodermoclysis; the establishing of a blood level, as in the use of the sulfonamides; are possible lines of experimentation.

4. Make physiological studies to determine the possible effects of toxin therapy upon the organism. The existing evidence indicates that certain beneficial effects may occur, including decided relief of pain, increase in appetite, better sleep, stimulation of wound healing, and regeneration of bone and other tissues destroyed by the neoplasm. These appear to vary according to the stage of the disease, the toxin used, the dosage, and the route of inoculation. The physiologic approach might include a study of the effects of toxin therapy on conditions other than malignancy, *i.e.*, arthritis, paresis, various eye affections, etc., conditions in which the toxins have been used empirically by various physicians other than Coley with apparent benefit.

5. Establish a central clearing house where detailed records of histories may be registered and progress analyzed. (For additional data address H. C. Nauts, 1290 Madison Avenue, New York 28, N. Y.)

SUMMARY

This study provides sufficient evidence, both clinical and experimental, to justify the conclusion that toxin therapy has clinical value, and that further extensive research is warranted in order to produce better preparations and further refinements in the technic of administration. Reasons are given to explain why the method has not achieved wider recognition in the past.

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Note: This is only a part of the total bibliography used in the course of making this analysis.

Abstracts

Reports of Research

The Ultraviolet Absorption Spectra of Aromatic Hydrocarbons. JONES, R. N. [Harvard Univ., Cambridge, Mass.] *Chem. Revs.*, **32**:1-46. 1943.

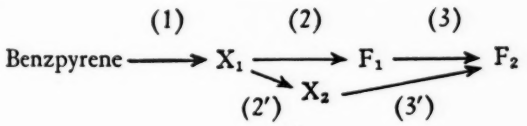
The ultraviolet absorption spectrum is of considerable value in the determination of the structure of aromatic hydrocarbons, including the carcinogens. The literature on this subject is reviewed, with 263 references, and 8 tables provide separate bibliographies of articles giving absorption spectrum data on each of 370 hydrocarbons. —M. H. P.

The Biochemistry of Benzpyrene. I. A Survey, and New Methods of Analysis. WEIGERT, F., and MOTTRAM, J. C. [Mt. Vernon Hosp. and Radium Inst., Northwood, Middlesex, England] *Cancer Research*, **6**:97-108. 1946.

After a review of existing knowledge of the metabolism of benzpyrene, methods are described for the separation, purification, identification, and estimation of 4 different products of the metabolic conversion. These products are designated by the symbols X_1, X_2, F_1, and F_2, and some of their properties are described.—Authors' summary.

The Biochemistry of Benzpyrene. II. The Course of Its Metabolism and the Chemical Nature of the Metabolites. WEIGERT, F., and MOTTRAM, J. C. [Mt. Vernon Hosp. and Radium Inst., Northwood, Middlesex, England] *Cancer Research*, **6**:109-120. 1946.

The metabolic conversion of 3,4-benzpyrene in mice passes through a number of stages, symbolized by X_1, X_2, F_1, and F_2 according to the following sequence:



The various metabolites are characterized by their fluorescence and absorption spectra and by their chemical chromatographic properties, which suggest their chemical constitutions.

The derivatives

$X_1 = 8(OR_1)\text{-}9(OH)\text{-}8,9\text{-dihydro-}3,4\text{-benzpyrene}$
$X_2 = 8(OR_1)\text{-}9(OR_2)\text{-}8,9\text{-dihydro-}3,4\text{-benzpyrene}$
and $F_1 = 8(OR_1)\text{-}3,4\text{-benzpyrene}$

have not been described previously, whereas F_2 is the known end product of the metabolism, 8-hydroxy-3,4-benzpyrene. After an intravenous inoculation of a finely dispersed colloid, the metabolism of 3,4-benzpyrene follows the sequence above in an approximately quantitative manner. The nature of the radicals R_1 and R_2 is not yet established, but they are derived from the structure of the cells with which the parent hydrocarbon and X_1 come into contact. The steps (1) and (2′) occur *in vivo* only, whereas (2) can be reproduced *in vitro* by a mild chemical reaction at room temperature, and (3) and (3′) by stronger agents at elevated temperature.—Authors' summary.

Effects of Massive Doses of Methylcholanthrene on Epidermal Carcinogenesis. STOWELL, R. E., and MÁAS, L. C. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research*, **6**:121-127. 1946.

Three groups of approximately 50 Swiss mice each were painted on the back with solutions of 1.0% methylcholanthrene 3, 6, and 9 times a week, while 2 other groups were used as unpainted controls and controls painted with pure benzene. Such tremendous amounts of methylcholanthrene produced malignant tumors a little more rapidly than smaller doses. As systemic effects of exposure to the methylcholanthrene the mice showed increased incidences of (a) inflammation in the liver, kidney, and lungs; (b) hyperplasia of the bone marrow, spleen, and lymph nodes with extramedullary myelopoiesis, lymphopoiesis and erythropoiesis; and (c) leukemia. It is not known whether these changes were brought about directly by the methylcholanthrene and its detoxification products, or indirectly through lowered resistance of the host to bacterial or viral agents. These results do not conclusively support either the theory that chemical carcinogens act directly by stimulating uncontrolled cell proliferation; or by a toxic action from which some surviving cells escape by adopting abnormal growth characteristics.

Some thyroid glands had atypical acini and some parathyroids were hypertrophic and hyperplastic. These experiments do not support the idea that such changes are primarily caused by the methylcholanthrene.—Authors' summary.

Calcium and Potassium Content of Secretions from Noncancerous and Cancerous Stomachs. DUNHAM, L. J., and BRUNSCHWIG, A. [Univ. of Chicago, Chicago, Ill.] *Cancer Research*, **6**:54-56. 1946.

Calcium determinations were carried out on juices from 14 patients with gastric tumors (10 carcinomas and 4 lymphosarcomas) and from 24 control patients. Differences in calcium could be explained on the basis of the higher acidity in the juices from control stomachs. Potassium determinations were carried out on the juices of 9 patients with gastric tumors (7 carcinomas, 2 lymphoblastomas) and of 11 control patients; no significant differences in the amounts secreted were observed. The secretion of both calcium and potassium was increased, compared with controls, in the juices of 5 patients with

achlorhydria induced by the x-ray treatment of duodenal ulcer.

The calcium and potassium concentration of gastric juice from cancerous stomachs is not characteristic. These negative findings are in contrast to significant differences observed between nonneoplastic and neoplastic gastric (and colon) mucosa, which will be described subsequently.—Authors' summary.

Isolation of Steroids from the Urine of Patients with Adrenal Cortical Tumors and Adrenal Cortical Hyperplasia, A New 17-Ketosteroid, Androstane-3(α),11-diol-17-one. Mason, H. L., and Kepler, E. J. [Mayo Foundation and Clin., Rochester, Minn.] *J. Biol. Chem.*, **161**:235-237. 1945.

A new steroid, androstane-3(α),11-diol-17-one, as well as several previously reported steroids, was isolated from the urine of 3 women with adrenal cortical tumors and from the urine of 4 patients with bilateral cortical hyperplasia.—H. J. C.

The Isolation of Pregnanediol-3α,17-one-20 from Human Urine. Lieberman, S., and Dobriner, K. [Memorial Hosp., New York, N. Y.] *J. Biol. Chem.*, **161**:269-278. 1945.

A new steroid, pregnanediol-3α,17-one-20, m. 219-219.5° C., considered to be a product either of an abnormal activity of the adrenal gland or of an abnormal metabolism of steroids of adrenal origin, has been isolated from the urine of a woman with adrenal hyperplasia, a woman with an adrenal tumor, a cryptorchid male, and a eunuchoid male injected with testosterone. This compound has not been found in the urine of normal individuals or of pregnant women.—H. J. C.

Betel Chewing Among Natives of the Southwest Pacific Islands. Lack of Carcinogenic Action. Eisen, M. J. [Med. Corps, A. U. S.] *Cancer Research*, **6**:139-141. 1946.

Betel chewing by the natives of New Guinea, New Britain, New Ireland, and the adjacent smaller islands does not appear to elicit cancer of the mouth.—Author's summary.

Intraperitoneal Sarcomas Produced in Mice with Mouse Ascitic Fluid. Herly, L. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research*, **6**:131-133. 1946.

Paraffin pellets containing 1 mgm. of methylcholanthrene, when introduced into the abdominal cavity of young adult male C57 mice, produced ascites in 50 days and sarcomas in all mice surviving for 90 days. The ascitic fluid, even when obtained before the development of sarcomas, produced ascites and malignant tumors when injected intra-abdominally into another series of mice of the same strain. The ascitic fluid obtained from this second series, when injected intra-abdominally into a third series of mice, again resulted in ascites and sarcomas. These tumors were transplantable by subcutaneous inoculation. Whatever the active agent may have been, it was destroyed by ether and by exposure to 90° C.—Author's summary.

Conjugated Forms of Myelokentric and Lymphokentric Acids. Turner, D. L., and Miller, F. R. [Jefferson Med. Coll., Philadelphia, Pa.] *J. Biol. Chem.*, **161**:91-97. 1945.

Myelokentric and lymphokentric acids, present in the urine of patients with leukemia, occur as the prosthetic groups of water-soluble conjugates. These conjugates, when administered separately to guinea pigs, produced myeloid and lymphoid lesions, respectively, and a mixture of them both produced lesions of the "Hodgkin's type."—H. J. C.

Attempted Transmission of Human Leucemia in Man. Thiersch, J. B. [Inst. of Med. and Vet. Sc., Adelaide, South Australia] *J. Lab. & Clin. Med.*, **30**:866-874. 1945.

The results were negative in numerous attempts to transfer human leukemias by injecting suspensions of leukemic cells derived from the blood, spleen, and lymph nodes of patients with chronic and acute myeloid and lymphatic leukemias into the subcutaneous tissues of other patients suffering from incurable diseases (cancer, heart disease) with a life prospect of less than 2 years. Results were negative also in attempts, made with whole blood, to cross-transmit chronic myeloid leukemia to patients with chronic lymphatic leukemia, and *vice versa*. The results are consistent with previous negative findings on the transmissibility of human leukemia, but the author suggests that more suitable recipients or a different tehnic might yield positive results.—J. G. K.

The Effects of Crude and Purified Penicillin on Continuous Cultures of Normal and Malignant Cells. Gey, G. O., Gey, M. K., Inui, F., and Vedder, H. [Johns Hopkins Univ. and Hosp., Baltimore, Md.] *Bull. Johns Hopkins Hosp.*, **77**:116-131. 1945.

Continuous cultures of normal (rat) and tumor (human, rabbit, and rat) [carcinoma and sarcoma] cells maintained in roller tubes as pure strains tolerate very high concentrations of penicillin sodium (Merck) for long periods of time. The results indicate that it is possible to cultivate almost indefinitely strains of cells in plasma culture media whose supernatant fluid contains penicillin sodium in concentrations of 5,000 Oxford units per cc. Such successful cultivation apparently depends upon the character of the immediate cultural environment as well as the inherent tolerance of the cell strain. No conclusive evidence of an increased tumor cell susceptibility to purified penicillin when compared to normal cells is justified from the results obtained on continuous cultures and on primary explants of tumors produced by inoculating continuous cultures of tumor cells into animals. The toxicity of crude penicillin filtrate is much greater than that of the therapeutic penicillin sodium and is perhaps due in large part to the toxicity of the mold medium from which the filtrate is made.—Authors' summary. (J. G. K.)

Neutralization of Inhibition of Tumor Growth. Keresztesy, J. C., Laszlo, D., and Leuchtenberger, C. [Merck and Co., Inc., Rahway, N. J., and Mt. Sinai Hosp., New York, N. Y.] *Cancer Research*, **6**:128-130. 1946.

By adapting a method that detects inhibitors of tumor growth it is possible to demonstrate that the action of inhibitors upon sarcoma 180 in mice can be effectively neutralized by both structurally related and unrelated substances. Neutralization by approximately equal amounts of inhibitor and antagonist was observed in the inositol—*p*-aminobenzoic acid, inositol—pyridoxine, and *d*-desthiobiotin—*d*-biotin experiments. Thiamin, niacinamide, *o*- and *m*-aminobenzoic acid, and leucopterin were slightly

active, if at all, in counteracting the inhibition caused by inositol. Interference could be detected when larger doses of some of these substances were given. While both *d*-desthiobiotin and an avidin concentrate were effective inhibitors of tumor growth, neutralization occurred when these two materials were tested for antagonism. Impurities in the avidin concentrate may be responsible for this interference.—Authors' abstract.

Histologic Changes in the Central Vegetative Centers of the Hypothalamus in Carcinoma as an Indication of Vegetative Functional Disturbances. MORGAN, L. O. [Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio] *Cancer Research,* **6**:142-147. 1946.

A histologic study was made of 5 nuclei of the hypothalamus in 19 patients with carcinoma of various organs. Extensive chromatolysis and cell destruction indicate that all these cell groups are involved in carcinoma. The pattern of these cell changes shows a wide range of variation. A congenital overdevelopment of some of the nuclei was indicated. The cell destruction that occurs in carcinoma makes it impossible to evaluate this factor properly. The 5 nuclei studied are regarded as constituting a central mechanism for the control and integration of vegetative functions. This control is mediated largely through the autonomic and endocrine systems and influences most if not all metabolic functions. The cell changes in the hypothalamus suggest a widespread but variable instability or irregularity of vegetative functions in the patient with carcinoma. This is in keeping with the finding of numerous investigators who have made functional studies in experimental animals or in cancer patients.—Author's abstract.

Melanosarcoma and Rhabdomyoma in Two Pine Snakes (Pituophis melanoleucus). BALL, H. A. [Biol. Research Inst. of San Diego Zool. Soc., and San Diego Co. Gen. Hosp., San Diego, Calif.] *Cancer Research,* **6**:134-138. 1946.

Malignant melanomas occurring in a male and female pine snake are reported. The primary tumor in the female snake arose at the margin of one of the large pigmented areas of the skin of the tail. Metastatic tumors were found in the liver and the celomic cavity. In the male snake 2 large melanomas occurred on the upper lip, and another tumor, a typical rhabdomyoma, sprang from the hard palate. These tumors appear to be the third or fourth instances on record of malignant neoplasms in snakes.—Author's abstract.

Biochemical Genetics. BEADLE, G. W. [Sch. of Biol. Sc., Stanford Univ., Calif.] *Chem. Revs.,* **37**:15-96. 1945.

This review, with 354 references, discusses the evolution, structure, and action of genes, the characters controlled by genes (including cancer), the chemical nature of chromosomes and genes, spontaneous and induced gene mutation, and viruses and plasmagenes. Five pages are devoted to the relationship between genes and cancer.—M. H. P.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

HEREDITY

Tumors in One of Homologous Twins. Hodgkin's Disease with Primary Skeletal Manifestations. CHARACHE, H. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Am. J. Roentgenol.,* **54**:179-181. 1945.

This is a report of Hodgkin's disease in one of homologous twins, who died at 5 years of age. The surviving twin was apparently normal $4\frac{3}{4}$ years after the onset of symptoms in the deceased twin.—E. H. Q.

RADIATION

Skin and Lip Cancer. SLOBODIN, H. [Vet. Admin., Hines, Ill.] Report to Chicago Roentgen Soc., Apr. 12, 1945. From abstr. in *Proc. Inst. Med. Chicago,* **15**:361-362. 1945.

Fractionated roentgen ray treatment, usually completed in less than 3 weeks, was successful in 225 skin carcinomas except for 3 recurrences, 2 irradiation ulcers, and 2 lesions that required supplementary surgery. Of the 3 recurrences, 2 were presumably controlled by subsequent surgery, and 1 patient died of extensive squamous carcinoma of the neck. Treatment was successful in all carcinomas about the eye and ear. In a series of 81 patients with carcinoma of the lip similarly treated, none showed local recurrence 2 years later, 3 developed cervical metastases, and 2 developed irradiation ulcers, which healed promptly. Of 6 patients with carcinoma of the lip treated surgically, none had local recurrences; 1 had extensive recurrent cervical metastases after neck dissection.—M. E. H.

The Response to Preoperative Irradiation as a Clue to the Management of Breast Cancer. LEVI, L. M. [Los Angeles Co. Hosp., Los Angeles, Calif.] *Am. J. Surg.,* **68**:355-357. 1945.

One hundred and thirty-one patients with breast carcinoma were treated with x-ray irradiation. Fifty-three showed conspicuous regression in the size of the mass, and of these, 29 were then subjected to radical mastectomy with a survival rate of 45%. In the remainder of the group in which the tumor regressed following x-ray but surgery was not employed, the survival rate was 56%. No consistent correlation was found between the histologic appearance and the response of the tumor to radiation. The results indicate that in highly radiosensitive breast tumors, irradiation alone delays metastasis, while subsequent surgery is prone to disseminate the disease: the average time after treatment to the appearance of metastasis was 16.22 and 10.77 months, respectively.—W. A. B.

NERVOUS SYSTEM

Glioma of the Optic Chiasma. MINTON, J. *Proc. Roy. Soc. Med.,* **38**:566. 1945.

Description of a case.—E. L. K.

Intracranial Lipoma. A Report of Four Cases. VONDERAHE, A. R., and NIEMER, W. T. [Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio] *J. Neuropath. & Exper. Neurol.*, **3**:344-354. 1944.

Four cases of intracranial lipoma are reported. Three of the tumors were found between the infundibulotuberal region and the mammillary bodies. Two of the lipomas were hemangiomatous in type, and one presented a collection of bipolar neurones. When the tumors are arranged in the order of ontogenetic progression, the basic similarity together with the differences are readily understood. There is a transition from simple lipoma to the more complex teratoid tumors and teratomas. Intracranial lipoma is probably never diagnosed in life. However, the present study indicates that these tumors may produce pressure effects and may invade the contiguous cerebral tissue. For this reason, intracranial lipomas must be reckoned with as possible determinants of some clinical manifestations.—A. Cnl.

Tumors in the Spinal Cord in Childhood II. Analysis of the Literature of a Subsequent Decade (1933-1942); Report of a Case of Meningitis Due to an Intramedullary Epidermoid Communicating with a Dermal Sinus. HAMBY, W. B. [Univ. of Buffalo Sch. of Med., and Buffalo Children's Hosp., Buffalo, N. Y.] *J. Neuropath. & Exper. Neurol.*, **3**:397-409. 1944.

In a survey of the literature of 1933, reports were found of 100 cases of intraspinal tumors in children of 15 years or younger. In the subsequent decade (1933 to 1942 inclusive), 114 such cases were reported. The most frequent tumors [among the total 214 cases] were gliomas (20%), sarcomas (19%), dermoids (16.7%), and neurinomas (10.4%). The incidence of some tumor types apparently has changed in these two time periods. A case is reported of a child having an infected epidermoid tumor extending from a dermal sinus into the congenitally elongated spinal cord.—Author's summary. (A. Cnl.)

Cutaneous Tumors of Von Recklinghausen's Disease (Neurofibromatosis). MCNAIRY, D. J., and MONTGOMERY, H. [Mayo Foundation and Clin., Rochester, Minn.] *Arch. Dermat. & Syph.*, **51**:384-390. 1945.

Fifteen cases were studied, in 12 of which nonmedullated nerve fibers were clearly demonstrated.—J. G. K.

Blood Vessels

Hemangioma of the Hand. SPEED, K. Report to Chicago Surg. Soc., Feb. 2, 1945. From abstr. in *Proc. Inst. Med. Chicago*, **15**:336. 1945.

Three case reports. The differential diagnosis of these tumors is not easy and must exclude many afflictions of tendons and nerves. Radium and x-ray are probably useless for complete cure. Surgical excision must be radical and nondestructive. The histology must be studied to detect the malignant changes.—M. E. H.

Bone, Bone Marrow, Joints

Further Studies on the Diagnosis of Bone Tumors by Aspiration Biopsy. SNYDER, R. E., and COLEY, B. L. [Memorial Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **80**:517-522. 1945.

The results of 567 aspiration biopsies of bone were studied and tabulated. Eighty-two per cent provided material for the diagnosis of tumor. There were no immediate complications of the procedure and no evidence to suggest more rapid development of metastasis following its use.—J. G. K.

Benign Bone-Forming Tumours of the Jaws. WILKINSON, F. C., and POLLAK, E. [Univ. of Manchester, Manchester, England] *Brit. Dent. J.*, **77**:341-346. 1944.

Descriptions of cases.—E. L. K.

Benign Giant-Cell Tumor of the Patella. ROEMER, F. J. [Vancouver, Wash.] *Am. J. Surg.*, **67**:563-566. 1945.

A case report. The age incidence, percentage of recurrence and of metastases, and the method of removal of the tumor in 21 cases gathered from the literature are tabulated.—W. A. B.

Solitary Eosinophilic Granuloma of Bone. GREENBERG, B. B., and SCHEIN, A. J. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Surg.*, **67**:547-555. 1945.

Two cases of solitary eosinophilic granuloma are reported: one lesion was in the clavicle of a boy of 15 and the other in the proximal metaphysis of the tibia of a $2\frac{1}{2}$ year old boy. In the first case, resection was carried out, and in the second, curettage was used; x-ray therapy was given postoperatively in both.—W. A. B.

Lumbar Vertebral Chordoma. ROBBINS, S. L. [Boston City Hosp., Boston, Mass.] *Arch. Path.*, **40**:128-132. 1945.

Case report.—J. G. K.

Malignant Tumors Arising from the Synovial Membrane with Report of Four Cases. MORETZ, W. H. [Univ. of Rochester Sch. of Med. and Dentistry, Strong Memorial Hosp., and Rochester Municipal Hosp., Rochester, N. Y.] *Surg., Gynec. & Obst.*, **79**:125-132. 1944.

A review of the literature, report of 4 cases, and clinical discussion.—J. G. K.

Cancerous Synovial Tumors. HARTZ, P. H. [Pub. Health Service, Curaçao, Netherlands West Indies] *Arch. Path.*, **40**:88-93. 1945.

A pathological study of 3 cases. In 2 cases, the tumor originated in the knee joint; in 1 case, in the foot.—J. G. K.

Pancreas

Radical Pancreatoduodenal Resection for Adenocarcinoma of the Head of the Pancreas. ERB, W. H. [Univ. of Pennsylvania, Philadelphia, and Taylor Hosp., Ridley Park, Pa.] *S. Clin. North America*, 1370-1376. 1944.

Discussion and report of a case. A successful radical pancreatoduodenal resection is recorded in which the blind end of the duodenum was utilized for anastomosis to the gall bladder.—J. L. M.

Partial Duodenopancreatectomy. Its Use in the Treatment of Pancreatic Malignancy—Case Report. LEE, H. C. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **72**:333-340. 1945.

The evolution of the operative procedure is described and discussed. In the case presented, the patient died on the 13th postoperative day of unknown cause. The authors believe it essential to preserve the pancreatic duct and implant it into the bowel if survival is to be maintained for any great length of time. Comparisons of pancreatectomized individuals with patients having congenital fibrocystic disease of the pancreas are made.—M. E. H.

Multiple Venous Thromboses with Associated Carcinoma of the Pancreas. FERRIS, E. B., and RITTERHOFF, R. J. [Cincinnati Gen. Hosp., Cincinnati, Ohio] *Ohio State M. J.*, **41**:437-440. 1945.

Report of a case. Many of the thrombi were apparently composed only of fused platelets and were present in both venules and arterioles, in contrast to the pattern of thrombosis of larger veins usually associated with pancreatic carcinoma. The occluded vessels did not contain tumor emboli, nor were they involved in an inflammatory process.—E. E. S.

Hyperinsulinism in Relation to Pancreatic Tumors. WHIPPLE, A. O. [Columbia Univ., New York, N. Y.] *Surgery*, **16**:289-305. 1944.

The syndrome of hyperinsulinism due to islet-cell tumor of the pancreas has been seen in 27 patients at the Presbyterian Hospital. Surgical excision rather than conservative therapy is advocated for 3 reasons: (1) On conservative treatment patients become too obese for later surgery. (2) Mental deterioration is frequently associated with repeated episodes of hypoglycemia. (3) Many islet-cell tumors are malignant. The surgical technic for removal of these tumors is outlined. A review of the literature since 1929 shows 106 tumors found at autopsy or operation that were considered benign, 28 tumors that were suspected of being malignant, and 15 cases of proved malignancy with metastases.—W. A. B.

Tumors of the Islands of Langerhans. RABINOVITCH, J., and ACHS, S. [Jewish Hosp., Brooklyn, N. Y.] *Arch. Path.*, **40**:74-77. 1945.

A report of 4 cases and discussion.—J. G. K.

Islet Cell Tumors of the Pancreas. BRESLIN, L. J. [Toronto, Canada] *Canad. M. A. J.*, **53**:160-162. 1945.

In this case, the chief presenting symptom was obstructive jaundice of fairly long duration. On exploration the case proved to be one of islet-cell carcinoma of the beta type that invaded not only the body but the head of the pancreas and a regional lymph node. The patient manifested signs of dysinsulinism.—M. E. H.

PARATHYROID

Functional Parathyroid Tumors and Hyperparathyroidism. Clinical and Pathologic Considerations. ALEXANDER, H. B., PEMBERTON, J. DEJ., KEPLER, E. J., and BRODERS, A. C. [Mayo Clin., Rochester, Minn.] *Am. J. Surg.*, **65**:157-188. 1944.

A review of the literature and a report of 14 cases of parathyroid tumor producing hyperparathyroidism. In 13 of the 14 cases, the tumor showed cytologic evidence of malignancy. In 12 patients the growth was removed surgically with complete relief of symptoms. The gross and microscopic pathological changes and the divergent clinical pictures are discussed, and the laboratory methods available for diagnosis evaluated.—W. A. B.

Osteitis Fibrosa Cystica: Differential Diagnosis. FOX, N., and TAGLIA, V. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Otolaryng.*, **37**:377-390. 1943.

A case report. Abnormal blood calcium and phosphorus levels returned to normal after removal of a parathyroid tumor.—W. A. B.

Severe Osteitis Fibrosa Cystica with Parathyroid Tumor. COBURN, D. E. [Fitch Clin., St. Johnsbury, Vt.] *Am. J. Surg.*, **66**:252-258. 1944.

A report concerning a woman of 60, who had had symptoms, chiefly pathological fractures, for 15 years before the finding of a parathyroid adenoma in the superior mediastinum.—W. A. B.

PINEAL

Pinealoma. DUBLIN, W. B. [Emery Cancer Clin., Los Angeles, Calif., and Tacoma Gen. Hosp., Tacoma, and West. State Hosp., Ft. Steilacoom, Wash.] *Northwest Med.*, **44**:86-87. 1945.

A case report. The tumor was thought to have been present from birth, producing internal hydrocephalus. A brief review of the literature on the clinical and pathologic features of this tumor is included.—E. E. S.

The Endocrinologic Aspect of Tumors of the Pineal Gland. DAVIDOFF, L. M. [Jewish Hosp. of Brooklyn, Brooklyn, N. Y.] *Surgery*, **16**:306-314. 1944.

Cases of pineal tumor are rare and in those reported there has been either precocious pubertal development or no endocrinologic disorder. An instance of the former type, occurring in a 9 year old boy, is reported. The theory that the pineal secretion initiates rather than inhibits puberty is discussed.—W. A. B.

MULTIPLE TUMORS

Multiple Carcinomas. A Case of Four Consecutive Primary Carcinomas with Apparent Cure. HOLLAND, C. A. [Temple Univ. Hosp., Philadelphia, Pa.] *J. A. M. A.*, **128**:356-359. 1945.

A case report. Four metachronous primary cancers occurred in different organs of the body in the same patient within a period of 10 years. These were adenocarcinoma of the right breast, squamous cell carcinoma of the esophagus, adenocarcinoma of the transverse colon, and basal cell carcinoma of the left cheek. All apparently have been cured, 2 by radical surgery and 2 by roentgen therapy. This is one of the first recorded cases of 5 year cure following roentgen irradiation for carcinoma of the esophagus. —M. E. H.

Multiple Primary Malignant Neoplasms of the Rectum and Sigmoid Colon. BACON, H. E., and GASS, O. C. [Temple Univ. Med. Sch., Philadelphia, Pa.] *Am. J. Surg.*, **68**:240-249. 1945.

Five new cases of multiple neoplasms of the rectum and colon are presented, and cases in the literature are reviewed and tabulated to show sites of the independent neoplasms. One hundred and thirteen references.—W. A. B.

MISCELLANEOUS

Important Factors in the Prognosis and Treatment of the Patient with Malignant Disease. JACOBS, A. W. [New York, N. Y.] *M. Rec.*, **158**:165-166. 1945.

General discussion.—E. E. S.

Some Long Shot Cases of Cancer that Recovered. HORSLEY, J. S. [Richmond, Va.] *Virginia M. Monthly*, **72**: 321-332. 1945.

The object of the paper is to show that in some instances

what appear to be hopeless cases may be cured or at least benefited. Among the 7 cases reported are 3 of mammary carcinoma, 2 of carcinoma of the stomach, 1 of carcinoma of the anterior mediastinum, and 1 of extensive basal cell cancer.—M. E. H.

Neoplasms Observed in an Army General Hospital. Present, A. J. [Hoff Gen. Hosp., Santa Barbara, Calif.] *Am. J. Roentgenol.*, **54**:47-53. 1945.

In the first 3,045 admissions to an Army General Hospital, 45 instances of neoplasm were found: 28 were benign, 17 malignant. The latter arose in bone, skin, lip, rectum and sigmoid, eye, brain, pancreas, and testicle. The range of age incidence was 20 to 58 years, the average being 29. Three especially interesting cases are discussed in detail: a dermoid cyst of the mediastinum, a teratoma of the mediastinum, and a fibroma of the stomach.—E. H. Q.

Case Reports of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis. Wood, W. B., Jr., and Moore, R. A. [St. Louis, Mo.] *J. Missouri M. A.*, **42**:146-151. 1945.

A case of malignant melanoma involving the brain, heart, lungs, kidneys, and other viscera is presented. The primary lesion may have been present in an eye removed 17 years previously for glaucoma.—M. E. H.

Bizarre Types and Locations of Lipomas. Caylor, H. D. [Caylor-Nickel Clin., Bluffton, Ind.] *Am. J. Surg.*, **67**: 530-535. 1945.

Two cases are reported in which lipomas simulated inguinal and femoral hernia. Other unusual lipomas described were in association with an epigastric hernia and with a thyroglossal duct cyst, and in locations causing pressure symptoms with pain radiating down the back. —W. A. B.

Neurocytoma of the Adrenal and Neuro-Epithelioma of the Retina. Wise, J. M. [City Hosp., Mobile, Ala.] *South. M. J.*, **37**:637-640. 1944.

The autopsy findings are presented for a case of neurocytoma of the adrenal with widespread metastatic involvement in a 2 year old girl, and a case of neuroepithelioma of the retina with metastasis to the central nervous system, in a boy of 3 years.—W. A. B.

Lymphangioma of the Abdomen. Murbach, C. F., Lewison, E. F., and Deibert, G. A. *Am. J. Surg.*, **68**:391-397. 1945.

Report of a case in a 35 year old male, in whom the tumor reached the massive weight of 18½ pounds but was not readily palpable because of its jelly-like consistency.—W. A. B.

STATISTICS

The Social Distribution of Cancer of the Scrotum and Cancer of the Penis. Kennaway, E. L., and Kennaway, N. M. [Roy. Cancer Hosp., London, England] *Cancer Research*, **6**:49-53. 1946.

One case only of cancer of the scrotum occurred in England and Wales in 30 years in 17 occupations of the highest professional class; this one case was that of a person who in earlier life had belonged to a lower social class. The number of cases to be expected among the same number of persons, not specially exposed to carcinogenic materials, in the general population would be about 22. Hence it appears that this form of cancer could be eliminated by social factors. As cancer of the penis does not show this social distribution these two types should not be pooled for statistical purposes. Data on the occurrence of cancer of the scrotum in native races and nonindustrial populations would be of great interest.—Authors' summary.

CANCER CONTROL AND PUBLIC HEALTH

Diagnosis of Cancer in a National Medical Service. Stebbing, G. F. [Lambeth Hosp., London, England] *Lancet*, **249**:65-68. 1945.

Proposals for the organization of the early diagnosis of cancer. "Cancer in this very early stage will only be recognised when it is specially looked for, and we must provide teams of highly trained and experienced specialists who will examine the patient with cancer in their minds. Such a team would have to examine a great many patients who were not suffering from cancer. It is therefore important that it should be composed of men who do not work in cancer alone. The idea of the cancer specialist has more than once been suggested, but I think it should be condemned. The team that examines the patient with these early symptoms must not only be able to decide that the patient has not got cancer, but must be able to decide what is causing the symptoms. Specialists of every kind must therefore be available for consultation with those who have to make the diagnosis. The team must work in a fully equipped hospital, and a large proportion of the patients will have to be admitted at least for a day or two. This will need a considerable number of beds. . . . .

"In many parts of the body a five-years survival-rate of more than 60% has been obtained where treatment is early and efficient, and this figure can probably be bettered when methods are further improved and facilities increased. Investigating a large number of patients attending my clinic, I find that the great majority have reported early symptoms to their doctors but have had inadequate investigation, symptomatic treatment, and discouragement from further attendance until some aggravation or complication has emphasised the need for further investigation. In other cases the general practitioner *has* recognised this need, but has allowed the matter to slide, perhaps for weeks or months before overcoming the difficulties in arranging the necessary investigation. There are still too many hospitals with a long waiting-list in which the patient has to take his turn. . . . .

"However perfect we can make the consultant and specialist services, it is important that the general practitioner should make as many examinations as possible himself. We must not come to look on the general practitioner as merely a signpost to a hospital special department. . . . .

"The relations between doctor and patient must be intensely personal, and this cannot be so if the doctor is a salaried servant of a public body, however that body may be constituted."—E. L. K.

Book Reviews

SIEBZEHN JAHRE STRAHLENTHERAPIE DER KREBSE. Zürcher Erfahrungen 1919-1935. [SEVENTEEN YEARS OF RADIATION THERAPY FOR CANCER. Cases from Zurich, 1919-1935.] Hans R. Schinz and Adolph Zuppinger. Leipzig: George Thieme, 1937. x + 340 pages. 95 illustrations and 213 tables. (Published and distributed by authority of the Alien Property Custodian, 1944.) Price $11.75.

This is a careful statistical analysis of the results of radiotherapy in patients with cancer treated in the radiological department of the University of Zurich between 1919 and 1936.

Emphasis is laid on the improvement in dosage and the results of radiotherapy during the period 1929 to 1936, as compared with 1919 to 1928. The 2,500 cases are classified according to the site of origin in 35 groups. The results of radiotherapy in each group are discussed in relation to age, sex, microscopic character of the growth, local peculiarities, extent of involvement on admission, and whether radiotherapy was the only treatment or was associated with surgery.

It is an excellent study, full of factual information of the results during that period.

MAURICE LENZ

DER MAGENKREBS. [GASTRIC CANCER.] George Ernst Konjetzny. Stuttgart: Ferdinand Enke. 1938. x + 289 pages. 155 illustrations and 20 tables. (Published and distributed by authority of the Alien Property Custodian, 1944.) Price $10.70.

The author records his researches on gastric carcinoma from the pathological viewpoint. For thirty years he has studied resection and postmortem material from the University Clinic in Hamburg and from neighboring cities. He stresses the importance of absolutely fresh material in order to demonstrate the changes he reports.

The book is divided into four sections, which cover the general pathology, etiology, pathologic anatomy, and histology of stomach cancer, and the clinical picture. It is amply illustrated. There are also 20 tables, and a rather large bibliography arranged alphabetically under the name of the authors at the back of the book. The writer states that he did not make this all-inclusive.

Under the first section, on general pathology, he reviews the frequency, age, and sex incidence of gastric cancer. Tables are shown that summarize these features from his material and from a few outside reports. This section comprises only 6 pages of the book.

The second section, on etiology, covers 100 pages. The author reviews the evidence for heredity; outside agents, including types of diet such as vegetarian; raw foods; alcohol; rural versus urban incidence of cancer; and the effect of the social status. Contagion, infection, and Cohnheim's cell-rest theory have nothing to support them as etiologic factors in gastric cancer. Trauma such as a burn may have significance only as a base upon which cancer may later develop. In fact the local process, whether following the short or long end stage of the inflammatory reaction, is most important: "Gastric cancer never develops on a healthy mucous membrane;" also, "Gastric cancer develops on a base previously prepared (by diseased tissue changes); that is, chronic inflammatory condition of the gastric mucosa or stomach wall." These changes Konjetzny calls precancerous, although some objection has been advanced against this term. The inflammatory changes of highest significance for the initiation of gastric cancer result from mechanical, chemical, thermal, infectious, and hematogenous stimuli, and progress from chronic gastritis, to chronic ulceration, to carcinoma. Gastric carcinoma is always preceded by an outspoken chronic atrophic gastritis or an atrophic hyperplastic gastritis. Under hypertrophic gastritis Konjetzny defines the changes by description and numerous photographs to illustrate his points. The usual postmortem material will not show these changes; the tissue must be fresh and from early cases, for advanced cancer is useless for demonstration of the fine details. Polyps of the stomach are always dangerous, for sooner or later they will develop malignant changes. In atrophic gastritis the alterations are found in the rugae and pits, where dark-staining, irregular epithelial cells may be found. Atrophic gastritis includes not only cases with complete loss of glands but also the progressive type. The pathological process advances through acute, subacute, and chronic changes.

Konjetzny discusses the theories of "age atrophy" and degeneration, as well as inactivation through failure of nerve stimuli. He also considers gastroscopic appearance, which he cannot correlate with the pathological picture, since the former necessarily represents "subjective interpretation".

He concludes that precancerous changes always precede carcinoma. They are present in small cancers where atrophic or hyperplastic gastritis can be demonstrated, for the malignant transformation originates not at one point, but in multiple foci over a broad surface of pathological mucosa.

He has not studied the relatively rare fundus and cardia cases, as his operative material has not been sufficient and he has had no opportunity to investigate early, small neoplasms. His conclusions are based on pyloric antrum carcinoma. He has no definite conclusions to draw on the heterotopic glands seen in these stomachs, and cannot state whether chronic gastritis has anything to do with them. He believes that the possibility of contiguous cells changing to carcinoma by "apposition" cannot be dismissed. He quotes the evidence for preceding gastritis, as shown by the atrophic gastritis of pernicious anemia with subsequent cancer. He shows by citing cases that cancer may arise in the areas of chronic gastritis at a distance from a chronic ulcer or in the stomach wall near an anastomosis.

He discusses the origin of cancer in chronic ulcer, and whether an ulcerating cancer was cancer from the first, or arose on an ulcer; it is impossible to decide by reports from the literature. In order to make the diagnosis a critical examination of all the evidence, including the histologic findings, must be made, for the question

whether any given lesion is cancer or simple ulcer cannot be settled by the history or by clinical means alone. Even at operation a decision cannot be made. The measured size of the ulcer may be of some help, but he has seen ulcers up to the size of a saucer that were not cancerous. Ulcers in the prepyloric region are prone to be cancerous. He re-emphasizes the chronic gastritis-ulceration-cancer sequence, but admits that the ulcer stage may be skipped in some cases. The same factors prevail for carcinomas that arise in the scar of an ulcer. As to whether the gastritis of ulcer can be differentiated from the gastritis in cancer, he feels that gastritis is not specific for either ulcer or cancer.

In the third section Konjetzny reviews the pathologic anatomy and histology of gastric carcinoma; this covers 48 pages. He considers the macroscopic picture and recognizes four main types with subdivisions. Multicentric origin is the rule. Metastasis to the stomach, which takes place by way of the blood stream or the lymphatics, is described and tabulated. The sharp edges and the situation in the submucosa help to make the diagnosis. Primary stomach cancers combined with cancers or benign tumors of other organs are discussed. The site of primary gastric cancer is in the pylorus in over 50 per cent, and nearer 75 per cent from his own observations. The microscopic picture is presented. Carcinoids may be seen in the stomach, but are rare. The question of a relationship between histologic appearances and prognosis or clinical behavior is considered; Konjetzny can find no significance in Broder's gradings in his own cases. The lymphatic drainage of the stomach is well pictured, and the relationship of gastric cancer to neighboring organs by direct extension shown by photographs. The duodenal edge is overstepped many times by gastric carcinomas, also the esophageal edge. Other complications such as perigastric abscess, peritonitis, and empyema receive attention. Metastases via the lymphatics, blood stream, or across the peritoneum are tabulated, and the predominent lymph node involvements, both regional and distant, well covered. Both common and rare metastases are enumerated, and this whole section, indeed, is a well rounded presentation of the pertinent pathologic picture.

The fourth section covers the clinical aspect; it is about 70 pages in length. In history taking the physician should be concerned with even the most remote symptoms, for if one waits for the textbook picture an early diagnosis will never be made; the possibility does not exist, since these is no such thing as an early carcinoma on a healthy stomach wall. The relation of chronic gastritis to cancer has not been considered exhaustively enough in the past. The physician should look for gastritis and be aware of the possibility that it may progress to cancer. The signs and symptoms are given and operative treatment, exploratory laparotomy, indications for surgery, and aftercare reviewed in detail. The resectability of gastric cancer has improved continuously from 1901 (18 per cent) to 1937 (58 per cent). Prognosis, operative mortality, surgical treatment, end results, 3 to 5 and 10 year survivals, recurrence, and treatment for inoperable cases are discussed with tables. This section is presented in an interesting fashion.

Throughout the volume Konjetzny expresses the view that gastric carcinoma is preceded by gastritis and ulceration. Whether this coincides with current pathological opinion or not it is a forcible presentation of the gastritis theory, and the evidence is well documented. The book is worth consideration by all those who deal with gastric conditions, and should be available in clinics where gastric carcinoma is under investigation.

JOHN J. MORTON

CORRECTION

From a source that seemed reliable at the time, but that turned out later to have been wholly unauthorized, the Editor received notice that Dr. Philip M. West wished to have his name omitted from the paper on "Demonstration of an Enzyme-Inhibiting Factor in the Serum of Cancer Patients (A Preliminary Study)" by Gregory Duboff and Samuel Hirshfeld (Cancer Research, **6**:57-60. 1946). The authorship of the paper should read: Samuel Hirshfeld, M.D., Gregory Duboff, M.S., and Philip M. West, Ph.D., M.D.

It now appears that Dr. West fully expected recognition as a coauthor, an acknowledgment to which he had every right as he was largely responsible for perfecting the chemical technic involved.

The Editor regrets that Dr. West should have been deprived of the credit that was his due.